ESSENTIALS OF LABORATORY DIAGNOSIS

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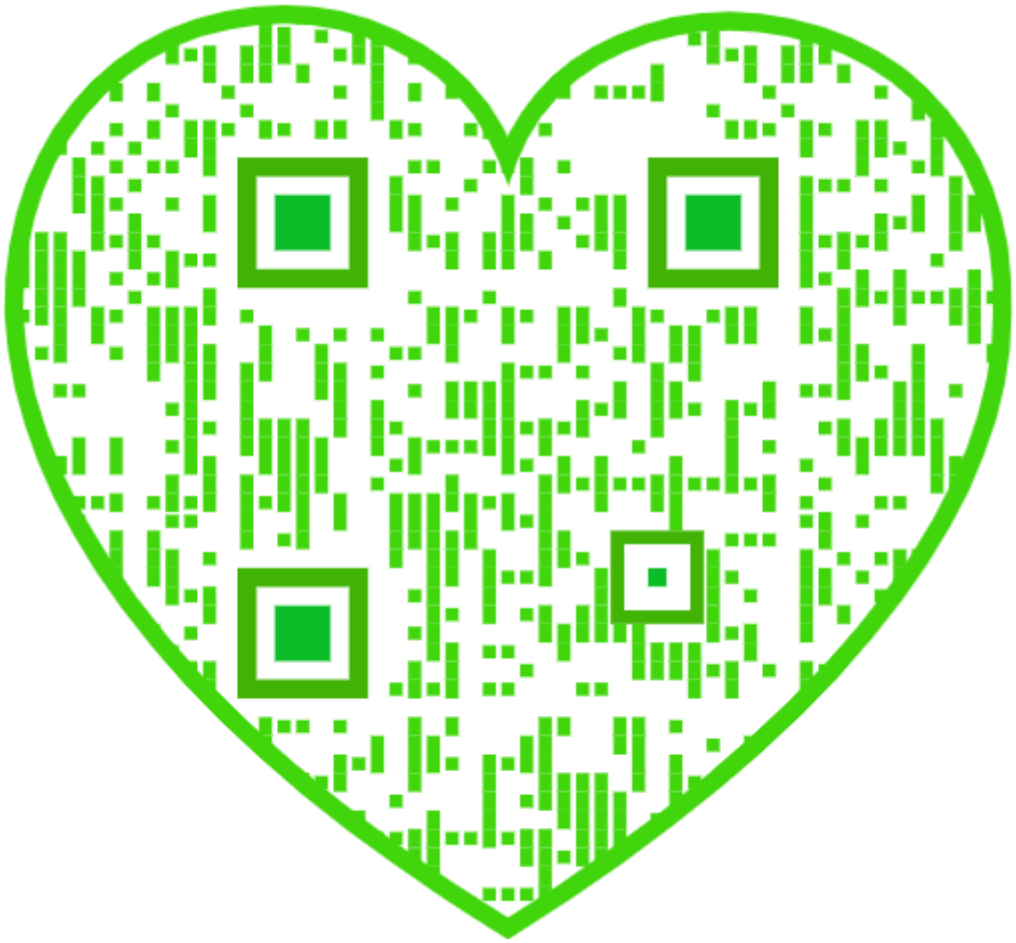

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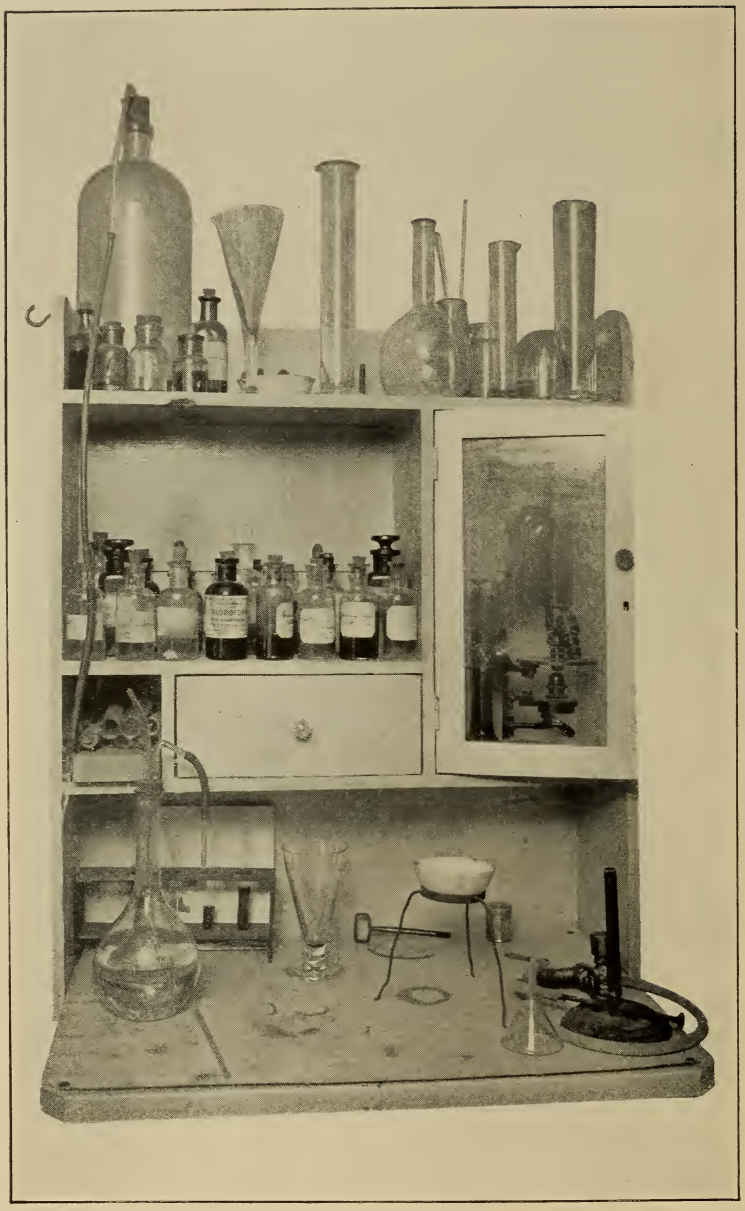

Author's Office Laboratory.

For details and description see Appendix, page 274.

ESSENTIALS

OF

LABORATORY DIAGNOSIS

DESIGNED FOR
STUDENTS AND PRACTITIONERS

BY

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CONTAINING AN INDICAN SCALE IN COLORS, SIX FULL-PAGE PLATES AND NUMEROUS ENGRAVINGS IN THE TEXT

PHILADELPHIA:
F. A. DAVIS COMPANY, PUBLISHERS
1909

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PREFACE.

A KNOWLEDGE of so many branches of medicine is required of the medical student of to-day, and as the time at his command is comparatively limited, a manual embodying the essentials of the subject such as this aims to do cannot but prove useful. At the same time, it is believed to contain all information necessary to provide a working outline of clinical laboratory methods for the busy practitioner.

The book is not intended to take the place of the many excellent and exhaustive text-books on Clinical Medicine, but rather to supplement them, by pointing out to the busy student and practitioner simple and reliable methods by which he may obtain the information desired, without unnecessary expenditure of valuable time upon difficult, tedious or untried methods.

The author has endeavored in this work to present in as concise a manner as possible a selection of the analytical methods employed in the clinical laboratory, without burdening the reader with unnecessary detail, cumbersome methods, etc., many of which are extremely difficult, often requiring considerable knowledge of general chemistry and elaborate apparatus, which may place them beyond the reach of the practitioner.

For these reasons it is hoped that this little work will prove equally valuable to the student and the practicing physician.

In preparing this work special effort has been made to bring the subjects treated up to date, by the introduction of such new methods as have proven reliable. Some of the

material is entirely new, and many of the plates and cuts have been prepared from original drawings and photographs by the author. Special mention is to be made of the plate and drawings (Plate II, Figs. 17 and 18) of the normal relations of the stomach, which were prepared expressly for this work by Dr. George E. Pfahler.

The appendix has been arranged to furnish a working basis for the equipment of a clinical laboratory, at the same time affording reference for the preparation of stains, reagents, etc., mentioned in the text.

The leading authorities have been freely consulted, and much material has been obtained from such authorities as v. Jaksch, Sahli, Caillé, Grawitz, Krehl, Max Braun, Tyson, Abbott, Purdy, Remsen, and Holland.

The author takes this opportunity to express his appreciation of the many valuable suggestions received from Drs. Judson Daland and Wm. Egbert Robertson, and Mr. Geo. B. Johnson, of the F. A. Davis Company; also, to his associate, Dr. Francis J. Dever, for invaluable aid in the preparation of the manuscript and in correcting the proof.

F. A. F.

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Philadelphia.

INTRODUCTORY NOTE.

By Judson Daland, M.D.
Professor of Clinical Medicine, Medico-Chirurgical College, Philadelphia.

This book should be in the possession of every medical student and most practicing physicians. It contains, as its title implies, the essentials, and only the essentials of those procedures necessary to clinical laboratory diagnosis. The peculiar value of this book resides in the concise and practical manner in which each subject is treated and each test described, and the entire absence of all superfluous data.

This book is particularly well suited, not only to the medical student in the preparation of cases assigned to him for study, but also to practicing physicians, who have grasped the necessity of establishing a small laboratory in connection with every-day work. Those possessing such a laboratory will find it necessary to consult this book at short intervals. The more it is employed the more will its practical value be demonstrated. I unhesitatingly recommend this work to medical students, and trust that it will find a place in the laboratory of every practicing physician.

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I.

THE MICROSCOPE.

SELECTION OF THE INSTRUMENT.

In purchasing a microscope one should consider nothing but the best. This is true economy, for a cheap or inferior instrument is always an unsatisfactory one which, when once bought, is hard to get rid of, and if later discarded for a better instrument, represents a total loss. The possession of a good microscope is an absolute essential. Its services are required in almost every investigation of modern clinical diagnosis, and there is hardly a chapter in this work which does not call for its use. While the finest and most expensive instruments are still of foreign make, there are, nevertheless, many of American make which compare favorably with the imported instruments. An example of a good American instrument is shown in Fig. 1, and is one of a series manufactured by the Bausch and Lomb Optical Co.

CARE OF THE MICROSCOPE.

In handling any instrument it is necessary to see that it is always grasped by some one of its solid parts—the base or the standard. Some of the newer makes are provided with an aperture in the standard for this very purpose.

If the instrument is to be in daily use it should be kept under a bell jar or in a specially prepared cabinet built above the work table. (See Frontispiece.) Reference to Fig. 1 will illustrate the mechanism and the different parts with which one should become familiar before attempting to use the instrument. Efforts to use the microscope by any one unfamiliar with its different parts and adjustment should never be permitted, since careless handling may result in serious damage to the objective or other delicate parts.

DESCRIPTION OF THE MICROSCOPE.

Referring to Fig. 1, the various parts of the instrument about to be described may be located.

The Ocular, or Eye-Piece, consists of one or more converging lenses, the combined action of which is to magnify the

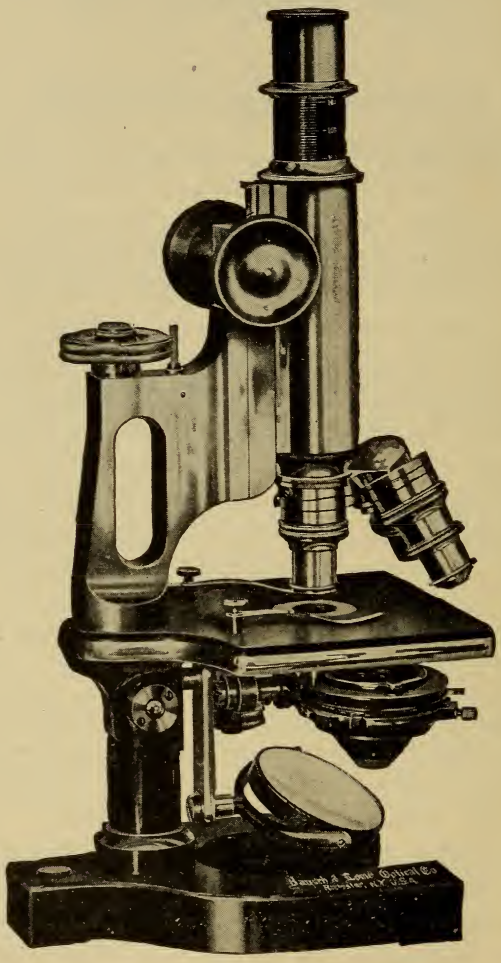

FIG. 1.—AMERICAN MAKE MICROSCOPE SHOWING TRIPLE NOSEPIECE AND APERTURE IN STANDARD TO FACILITATE HANDLING.

image formed by the objective. This part is contained in a tube of its own which telescopes into the top of the barrel, and is the part which the eye approaches when viewing the object.

Various oculars are usually provided, and are numbered from 1 to 8. As the number advances the magnifying power of the ocular increases; at the same time the length of the tube decreases. It is advised when increased magnification is desired, to accomplish this by increasing the power of the objective rather than by using stronger oculars. The clearness and definition of the picture will then be preserved, whereas if the power of the ocular is increased, it is usually at the expense of definition.

It will be found of great convenience to cement into one of the oculars a fine hair or eye-lash. This should rest upon the upper surface of the lower lens of the ocular, and will always be in focus. This serves as a pointer, and can be used to single out from a blood or other specimen some special point of interest, so that it can be shown to another person. This is particularly valuable in class demonstration.

The Barrel, or Tube, is the large tube of the microscope which serves as a conductor of the rays as they pass from the objective to the ocular.

The Objective consists of a system of converging lenses arranged at the lower end of the barrel, where it forms a magnified inverted image of the object. Upon this piece depends the magnifying power of the microscope. Most objectives are designated by numbers running from 1 to 15, these numbers representing the fractions of an inch at which the lens operates. Foreign makes are designated by letters which are not in any way directly comparable to the numbers of the American objectives. For one desiring three objectives, the most useful numbers will be found to be the 3, the 7 or 6, and the 12-oil-immersion, the 3 and 6 or 7 being chiefly employed in blood-counting, while the 12th is necessary for blood-examination and bacteriologic work.

The Stage is the table fastened below the barrel and in a right-angle plane to it. This serves to retain the object in a vertical plane to the optical axis of the instrument. The table is provided with spring clips to better hold the slide in position during examination. The newest Leitz scope is furnished with a movable (not mechanical) stage which, by the operation of two milled screws, is capable of slight movement in all direc-

tions. This, when a mechanical stage is not at hand, is of great service in searching blood and bacteriologic slides.

The Reflector is a small mirror situated below the stage which serves to direct the rays of light upward through the object in the optical axis of the microscope. The reflector has two sides, one carrying a concave, and the other a plane, mirror.

The Sub-Stage Condenser (Abbe's) consists of a system of lenses arranged between the stage and the reflector. The object of this part of the instrument is to collect and condense the rays as they come from the reflector, so that they are focused upon the object, thus furnishing brilliant illumination.

The Iris Diaphragm is now universally employed for controlling the intensity of the illumination. This is held in the same carrier as the condenser, and is just below it. By means of a lever every gradation of light, from the most intense to absolute darkness, is readily obtained. The proper manipulation of this diaphragm constitutes a very important part of the practical knowledge gained from the use of the microscope, and has much to do with the success of many investigations.

The Adjustments.—The *coarse* adjustment is the rack-and-pinion mechanism projecting from the upper part of the standard, and is employed to rapidly raise and lower the barrel and its attachments. The *fine* adjustment is a micrometer screw situated usually below the rack and pinion. This serves the purpose of very gradually raising or lowering the barrel in order to obtain exact focus.

The Draw-Tube is a very important adjunct of high-grade instruments, because by its skilful manipulation slight errors in refraction, due to inequalities in slide or cover-glass, may be corrected.

The Nose-Piece, or collar, is fastened to the lower end of the barrel, and permits the attachment of two or three objectives at one time in such a position that, by rotation of the collar, any one of them may be immediately brought into the axis of the instrument.

The most important accessories of the microscope are the objectives, and the quality of all microscope work largely depends upon their perfection. While the quality of objectives vary much, one cannot go far astray if they are obtained through

a reputable supply house or from a well-known manufacturer, such as the Zeiss or Leitz abroad, and the Bausch and Lomb Optical Co., U. S. A.

THE OIL-IMMERSION OBJECTIVE.

The oil-immersion objective, or homogeneous system, is so constructed that when in use the pencil of light passing through the object to the objective traverses only media of the same refractive index. This is accomplished by placing between the cover-glass and the end of the objective a medium having the same refractive index as glass. To accomplish this a drop of cedar oil is placed upon the cover-glass, and the objective brought into contact with this, and the observation made through the oil. This class of lens is intended to work only with the oil, and is unsatisfactory when used dry. Frequently it is not convenient to use cedar oil for the preliminary examination often employed to determine the progress of staining, etc.; here the staining fluid may be washed off and a drop of water used in place of the oil; this will give a sufficiently good picture for the purpose, and has the advantage of not interfering with the addition of further stain when desired.

The tube or barrel of the microscope is made in two forms, the long or English type being from 8 to 10 inches, and the short or Continental type having a length of 6½ inches. The latter is the more desirable form, since the majority of manufacturers of objectives adjust them for the short tube.

APOCHROMATIC OBJECTIVES.

This term is applied to a particular variety of lens containing a special kind of glass (containing calcium fluoride), besides the usual crown and flint glass, the object of this being to produce a greater degree of achromatism, thus reducing chromatic aberration. The special value of these objectives when used with a compensating ocular (compensating eye-pieces are specially constructed for use with apochromatic objectives), is as follows: Three rays of the colored spectrum, instead of two, as in case of the achromatic glasses, are focused in the same plane, leaving only a minute tertiary spectrum; also with these objectives the spherical aberration is corrected for two

colors in the brightest part of the spectrum, and the objective shows the same correction for marginal rays as for the central part of the aperture. Finally, apochromatic objectives admit the use of highly magnifying oculars. This form of objective is particularly useful in photomicrographic work, where it is very important to abolish chromatic aberration.

ILLUMINATION.

Illumination constitutes a most important factor in the practical use of the microscope, and upon its proper management depends very much of the efficiency of the work. Direct unmodified sunlight is unsuited, and should not be employed for general work. North light is the most uniform and steady, and is to be preferred. The use of artificial light should, as far as possible, be avoided, and when it must be used its character and color may be greatly improved by inserting a piece of blue glass between the reflector and the object. It is always necessary to keep the eye close to the ocular in an endeavor to keep the unemployed eye open. Too much light is always to be avoided, as it is only an added strain, accomplishes nothing, and may materially interfere with the efficiency of the work.

Oblique Light.—For urinary work the oblique light is best. By this term is meant light in which the parallel rays from the plane mirror meet the optical axis of the microscope at an angle. This form of light may be obtained in the following ways: *(a)* By placing the reflector to one side of the stage, this results in an oblique ray without materially lessening the strength of the light; the condenser must, of course, be swung out of the way. In microscopes which have a fixed mirror, *i.e.*, only movable perpendicularly at right angles to the axis, oblique light may be obtained through the condenser, as follows: *(b)* First, focus the light upon the object through the condenser; then, lower the condenser until its focus is considerably below the plane of the object, thus the rays emerging from the condenser will decussate and then diverge, so that all but axial rays will fall upon the object in an oblique direction.

TO CLEAN THE MICROSCOPE.

Should the lenses or oculars become blurred or spotted from dust or dirt, proceed as follows: If the dirt is upon the ocular it will be discovered by rotating the ocular within the barrel while observing the illuminated field of the microscope. If the obstruction is upon the ocular it will be seen to move; if upon the objective it will remain stationary during this manipulation. To find an obstruction upon the objective rotate it, when the spot or mark will move with it. Finally, if after testing both the objective and ocular in the manner described above the location of the dirt or dust is not demonstrated, the trouble will then be found upon the glasses or spectacles if the observer happens to wear them. Having located the trouble, remove the affected part and cleanse as follows: If careful polishing with bibulous paper or fat-free silk fails to accomplish the desired result, then moisten the silk or paper with a trace of distilled water, using this to aid in the removal, finally polishing dry. For oily or resinous smears a small amount of alcohol may be used. Special care should then be exercised to avoid any excess of the solvent which might enter between the individual lenses and, dissolving the cement, ruin the part. After using the oil-immersion it should always be cleaned before leaving the instrument. After removing the excess of oil with the silk, wipe off the remainder with a trace of xylol or benzine, immediately wiping dry. The cover-glass preparation may be treated in a similar way.

Glass surfaces should never be touched with the fingers, and great care should be exercised to avoid dropping and possible fracture of the oculars and objective. All *metal* parts of the instrument should be kept free from liquids, particularly acids and alkalies, benzine, xylol, alcohol, turpentine, and chloroform.

To clean the mechanical parts, none but the best machine oil should be used, and this only in small amounts and at long intervals. The polished brass requires nothing but occasionally polishing with clean chamois.

The two keynotes to successful use and preservation of the microscope are: handle with care and keep scrupulously clean.

THE MECHANICAL STAGE.

Of the many aids to exact work in the realm of clinical medicine the mechanical stage is of great practicability and wide application. Every possessor of a microscope should aspire to the possession of a mechanical stage, for this mechanism is not only a great time saver, but will materially aid in the search for bacteria and pathologic cells in the blood, and is practically a necessity in making a differential count.

Description.—Referring to Fig. 2, the general appearance of this instrument will be seen. It is designed for application to the stage of the microscope, upon which it is rigidly fastened

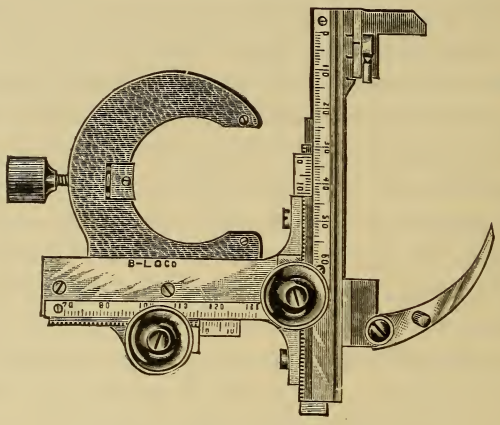

FIG. 2.—MECHANICAL STAGE.

by a collar and set screw attached to the stand. It is important in this connection to note that most manufacturers of microscopes make a mechanical stage for their particular instrument, and which frequently is not interchangeable with microscopes of other manufacture, so that, before purchasing, one should be certain that the stage will fit the instrument for which it is intended. When attached to the stand the slide carrier should move in both directions without any undue force and without any irregularity or jarring of the mechanism, and to the limits of its movement should remain in close and even contact with the microscope stage.

The mechanical stage is fitted with two milled screws which are movable in either direction, and which convey gradual

motion to the slide held in the jaws of the instrument. One of these screws controls the vertical, and the other the horizontal, motion, so that by proper manipulation it is possible to rapidly and accurately go over the whole of a specimen. (For more detailed description of the use of the mechanical stage in the differential count, see chapter on Blood.)

Most mechanical stages are provided with a millimeter scale and vernier reading to 0.1 of a millimeter, which serves not only to measure the size of small objects upon the stage, but is also very useful for locating objects of special interest upon any slide, so that they may again be found without difficulty. (See below the Maltwood finder and Pepper's application without the mechanical stage.)

THE WARM STAGE.

A further differentiation and improvement upon the mechanical stage is the stage prepared for preserving specimens at body-temperature during examination. This is provided with a thermometer which indicates the temperature of the object during examination. An extemporaneous warm stage has been described in another section (Blood Parasites) to which the reader is referred.

THE MALTWOOD FINDER.

In microscopic work, especially in studying blood or bacteriologic slides, some sort of "finder" is an essential part of the equipment. While the vernier scale, which is attached to most microscopes is fairly satisfactory for individual work, it does not fulfill all conditions demanded of it, since the same stage and microscope must always be used, and if by accident the relation between microscope and stage is altered ever so little, then all previous figures indicating location are rendered valueless.

The Maltwood finder (Fig. 3) does not possess the above disadvantages, and can be used universally with uniform results.

The Maltwood finder[1] consists of a heavy glass slide comparing exactly in size with the ordinary microscope slide. The central third of this slide is covered with a close network of

[1]Wm. Pepper: Jour. Amer. Med. Assoc., July 20, 1908.

intersecting rectilinear lines which form a large number of uniform squares. Each square contains two figures arranged one above the other, so that no two squares represent the same combination (see below). This marking has been placed upon the slide by a photographic process.

1 1	1 2	1 3	1 4	1 5
2 1	2 2	2 3	2 4	2 5
3 1	3 2	3 3	3 4	3 5

All Maltwood finders are made interchangeable, the squares coinciding exactly in all slides.

Method of Using the Finder.—If on looking over the slide with the mechanical stage a part of the field is discovered which

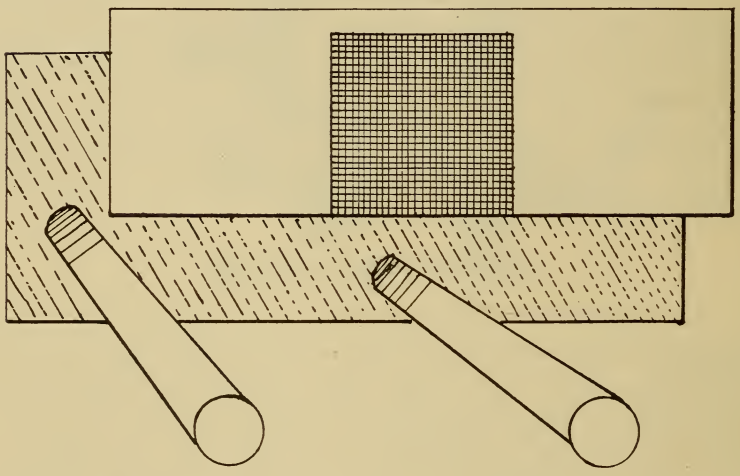

FIG. 3.—MALTWOOD FINDER, SHOWING DR. PEPPER'S BRASS ANGLE SUBSTITUTE FOR MECHANICAL STAGE.

one desires to examine at a later time, the slide is carefully removed without altering the location of the mechanical stage and the Maltwood finder substituted; the square is then observed which corresponds with the center of the slide. This combination is to be jotted down, and then in order to make sure that the proper reading has been made, the finder and slide should be substituted for each other a number of times. Sup-

pose this reading was made $\left|\frac{19}{21}\right|$, then, when later this particular field is sought the above process is reversed, and when the recorded square is in the center of the field, the slide is carefully substituted, when the object sought will appear in the field.

One of the chief advantages of the finder is that the slide may be sent to any one having a finder, with a note to look for such or such a square. For those not possessing a mechanical stage, Dr. W. Pepper has devised a small right angle of brass which may be easily carried in the pocket, and which may at any time be substituted for the mechanical stage, as the only purpose of the mechanical stage is to afford a fixed angle into which the slide and finder may be fitted.

The illustration shows the arrangement of slide and brass angle, and illustrates the size of the right angle compared to the slide. The strip of brass from which the form is cut should be about one-sixteenth inch thick.

EXAMINATION OF URINARY SEDIMENTS.

Preparation of the Slide.—After concentration of the sediment by centrifugation or sedimentation, the next point is to arrange this collection upon the slide for examination under the microscope. For this examination a few drops may be taken up in a pipette and allowed to fall upon the center of a clean slide, upon which a clean cover-glass is immediately placed. This will exclude dust, prevent rapid drying during examination, and will so flatten the field that frequent change in focus will not be necessary, while the different elements are more easily viewed and differentiated owing to their uniform separation and lack of overlapping.

The proper removal of the sediment from the bottom of the container is an all-important part of this process, and upon this the successful finding of casts will frequently depend. This is best accomplished with a small nipple-pipette or section of narrow glass-tubing, one end of which is drawn out into a coarse capillary point. Having cleansed the slide and cover slip, arrange them in a convenient place and then proceed to remove a few drops of sediment from the bottom of the tube. It is absolutely necessary, during this procedure, to prevent the entrance of an excess of fluid into the pipette. This end is accom-

plished in the case of the pipette by carrying it down into the fluid nearly to the sediment, then expressing a few bubbles of air before passing the tip beneath the sediment. With the straight tube the moist thumb or finger is held firmly upon the upper opening of the tube while its tip is carried to the bottom of the vessel, then by means of a slight rotation of the tube while relaxing the pressure above, a few drops of sediment are allowed to enter the tube. Firm closure of the upper end of the pipette is maintained while the tube is withdrawn and its contents transferred to the slide. To further guard against diluting the sediment, the outside of the tube should be wiped dry after it has been removed from the urine.

Special Technic for Excessive Sediments of Phosphates, Pus, etc.—It is not uncommon to meet with urinary sediments of such volume and density that the grosser condition may completely obscure the more important but less numerous elements, such as casts, pus, blood-cells, etc. In such cases the following technic is advised: After taking up the sediment deposit but one drop upon the slide; then from a clean pipette add a few drops of distilled water or, what is better, the clear urine from above the sediment. Agitate this till evenly mixed, and then apply the cover-glass and remove excess of fluid with strips of filter- or blotting-paper. Thus diluted not only is there better opportunity of finding casts, but the internal structure of the different elements will be much clearer.

Microscopic Search.—After preparation of the specimen as above outlined, the slide is transferred to the horizontal stage and first viewed by low power and a rather subdued light. In this examination it is safer to begin with the objective below the focal point, then with the eye in position gradually rack upward, at the same time keeping the slide moving with the other hand. If this is continued carefully there will be little difficulty in discovering the objects in the sediment, even if few and small. Another advantage of this plan is that all danger of crushing the slide or fracturing the objective by forcibly racking down is eliminated, since the objective is never carried downward except when the relation of objective to slide is plainly seen.

Having focused the sediment and regulated the light, the

slide should be searched as follows: Slowly move the slide forward until one corner of the cover-glass appears in the field, then locate the lower right-hand corner and gradually move the slide upward in as straight a line as possible, until the opposite border is reached. Then move slightly to the left and proceed in a straight line downward to the lower margin again. This procedure is repeated until the whole area of the cover-glass has been searched. For those having a mechanical stage the slide may be placed in this, and the same movements carried out with its aid. In either case it is well to keep one hand upon the fine adjustment to sharpen up any obscure objects which may be encountered.

Casts.—In searching for casts note any elongated structures which may appear in the field, and study carefully their outline under varying conditions of light and focus. Note carefully the fundamental characters of the body, whether granular or clear; if cellular elements are attached, such as epithelia, blood or pus, and if any of these are found whether they are well preserved as indicated by a sharp outline, or whether irregular and ragged from disintegration and age. Note also if there are any small, round, highly refractive granules (fat globules). Observe the relative size of the casts, as the predominance of certain sizes may indicate the location and character of the change in the kidneys.

There will be little difficulty in finding granular or cellular casts, as they are highly refractive and stand out plainly. Not so with almost invisible hyaline casts, which are very difficult to find on account of their feeble refractive powers. As aids to the detection of hyaline casts, it is important to remember that their outline is often accentuated by oblique light, and that too bright a light may absolutely obscure them.

Diagnosis of Casts.—The distinguishing features of casts under the microscope are: *(a)* Uniformity of marginal outline throughout the whole or greater part of their length, the edges stand out at all times as well defined borders which do not look accidental, but show the moulded effect of the kidney tubules. *(b)* For the most part casts are uniform in their individual diameters, and do not appear to suddenly bulge and then become as suddenly constricted. The body of the cast never appears

split, though it may occasionally be found curved and rarely twisted. *(c)* Casts are rarely exceedingly long, being usually between three and eight times their diameter or width. *(d)* The ends of casts are either rounded like the finger-ends or they are abrupt and ragged, plainly giving evidence of fracture; they never gradually taper off and disappear.

Diagnosis of Cylindroids.—These structures are very commonly met in the urine, and may become a source of more or less confusion and doubt. They are long, slender threads that have distinctive features which serve to distinguish them from casts. These distinguishing features are: *(a)* Their outlines are always more or less irregular, indistinct, and non-linear. *(b)* Their diameters are consequently very variable. *(c)* Their ends often taper into long, slender points which may be split, bifurcated or branched, but they never possess the smooth, rounded or abruptly fractured terminations of casts. They are rarely ever cellular or granular, but under high power frequently present longitudinal striations extending through the whole or a part of their length. *(d)* They are usually very long and slender, their total length frequently exceeding the diameter of the field when viewed through the medium power. *(e)* They are usually bent and often twisted into grotesque forms.

Epithelia.—Various forms of epithelia are found in nearly all specimens of urine, and careful note should be made of their form and size, as well as of the condition of their protoplasm and nuclei. They are highly refractive, and are therefore plainly visible under the microscope, their outlines standing out clear and distinct. The general character of the epithelia found corresponds to the usual three varieties found throughout the body, viz.: squamous or flat, cuboidal, and columnar or cylindrical. All epithelia are more or less granular and possess one or more nuclei, though the latter are not always visible, having been disintegrated or become obscured by granular degeneration of the protoplasm. Epithelia are subject to certain physical alterations when in the urine. By absorption of water they swell up and become more regular in outline, the small forms thus becoming spherical. The small cuboidal or columnar epithelia are most important, since they probably come from the tubules of the kidney. The larger varieties of

columnar and squamous cells come from the lower portion of the genito-urinary tract, the largest cells of all being the squamous vaginal epithelium.

Crystals.—Little difficulty should be experienced in recognizing and classifying the crystals found in the urine. For the most part they are comparatively large and highly refracting, and are therefore best viewed by moderate power and good illumination. One exception to this general rule occurs in the case of calcium oxalate, which may be very minute and exceedingly numerous. As a general rule, all crystals that show yellow or brown pigment are uric acid or urates, this characteristic serving readily to differentiate the group. The clear, large prisms (coffin-lid) are triple phosphates, while the smaller forms of highly refractive stars, crosses and dumb-bells are calcium oxalate. Reference to the plates in the section on urinalysis will show the character of the common, as well as of the rare, forms of crystals occurring in the urine.

Pus and Blood-Cells.—For the satisfactory study of these small elements, a higher power objective than that used for the study of crystals and epithelia is required. Oblique light, with moderate illumination, will be found best. Occasionally great difficulty is experienced in differentiating blood, pus, and small, round epithelial cells. This confusion is not so likely to occur when all varieties are present in sufficient quantity in one specimen to admit of careful comparison; but when only a few scattered cells of one variety are found, mistaken identity is quite possible. It is well, as a general guide, to bear in mind the relative size of these different elements. Pus corpuscles are the most common, and these should be thought of first. They are identified as small granular discs, having multi-nuclei. Somewhat smaller in size (about one-third) will be noted the pale, non-granular, non-nucleated discs which are the red blood-cells. While at least one-third larger than the pus cells, the so-called renal epithelia present a granular protoplasm with large nuclei.

Finally, after determining the individual elements in a given specimen, it is important to note the degree of degeneration and the presence of fatty change, particularly, occurring in the casts; and, lastly, the number of each formed element per

field, or per drop, should be determined. This observation being important in following the course of any pathologic condition, as it is similar to the repeated quantitative determination of albumin or sugar.

Micro-organisms.—The presence of excessive numbers of micro-organisms, their motility, and even some of their morphologic characteristics may be determined during the microscopic examination of the sediment; a complete and satisfactory examination of their detail and identity cannot, however, be determined except by special bacteriologic technic, for which the reader is referred to larger works on bacteriology or urinary diagnosis.

EXAMINATION OF THE BLOOD.

For an examination of fresh blood the 6 objective is most suited, since nothing but the cell outlines can be seen with the 3. A good light, with the condenser in close apposition with the under surface of the slide, will give the best results. To differentiate the varieties of white cells by their nuclei and granulation requires very careful regulation of the light, since the very slight difference in refraction of nuclei and protoplasm makes the differentiation extremely difficult at best.

Counting the Corpuscles: Red Cells.—Having made the proper dilution and mounted the specimen as outlined in the section on blood, the slide is placed upon the stage and a few minutes allowed to pass while the corpuscles settle to the bottom of the chamber. The medium power, 6, is brought into close apposition with the cover-glass, and then with the eye to the ocular the fine adjustment will bring the individual cells into view. Considerable difficulty is at times experienced in locating the part of the counting chamber containing the ruled lines. This may be overcome by centering the inner circle of the chamber by means of the outside of the objective. If this is carefully done the first attempt at focusing will usually bring the squares into view. Some hemocytometer slides are very faintly ruled, rendering the operation of counting very difficult. This may, in a measure, be overcome by reducing the light, at the same time taking advantage of the slight shadow caused by oblique rays coming from the lowered condenser. If this is done

great care should be observed in determining border-line cells on account of the uncertain shadow cast by all cells with this illumination.

The White Cells.—Either the 3 or the 6 objective may be used in this connection, and of these the 3 is generally preferred. It does, however, require a little more care and experience to make an accurate count, because of the minute appearance of the individual cells. Its chief advantage is that it is not necessary to move the slide while counting the entire field, after it has once been centered, thus doing away with the error of missed squares which occasionally occurs in moving the slide when using the 6 objective. Here, again, low illumination and oblique rays may advantageously be employed.

Examination of the Stained Specimen.—Since the usual reason for staining and examining the dried specimen is to study minute structural characteristics, to make a differential count or to discover the presence of bacteria or parasites, it is necessary to employ the oil-immersion lens and to occasionally supplement this with a highly magnifying ocular. In this work good illumination and sharp definition (condenser close to slide) are essential.

THE CAMERA LUCIDA.

The CAMERA LUCIDA is a device by the aid of which it is possible to quickly and accurately trace upon paper the magnified image of any microscopic object upon the microscope stage. It will be found of particular value in parasitology, where it is required to study in detail the minute anatomy of organisms too large to be viewed at one time under the microscope.

This apparatus is essentially a combination of mirrors and lenses by which the image of the sheet of paper is reduced by a suitable lens and is projected into the field of the microscope, so that the eye of the observer at the eye-piece of the camera lucida sees the image of the object and the paper and pencil of the examiner at the same time, *i.e.,* apparently the enlarged object has been transferred to the sheet of paper, so that the pencil, as followed by the eye, can be made to trace the lines of the object upon the paper.

The essential parts of the apparatus are the eye-piece, which

is arranged to clamp firmly above the ocular of the microscope in the optical axis of the instrument. This contains mirrors and lenses suitably arranged to reduce and deflect the image projected from the large reflecting mirror situated to the right of the ocular. This large mirror serves to reflect the image of the paper and pencil tip into the condensing lens of the camera lucida.

In setting up this instrument, the circular clamp on the camera lucida is firmly attached to the upper part of the ocular, and the horizontal bar carrying the reflecting mirror is clamped into the guide provided for that purpose. The camera lucida is so hinged to the clamp that it can be swung out of the way while locating the proper field in the microscope, after which it can be swung into place and centered by means of two small, set screws. The reflecting mirror is also arranged so that its angle can be adjusted between forty-five and eighty degrees.

II.

THE SPUTUM.

GENERAL CONSIDERATIONS.

Sputum, or expectoration, is voided by coughing or clearing the throat. It is composed of the secretion and the exudate from the mucous membrane of the nose, pharynx, and trachea, down to the finest bronchioles and alveoli; also of material that may have entered the respiratory tract from adjacent organs (pus of abscesses and empyema); blood derived from anywhere along the respiratory tract; and finally of material coming from the buccal cavity and from any part of the digestive tract.

On account of this very complex origin the composition of the sputum is very variable. The sputum may be present, but may not be expectorated. Small children and occasionally adults, on account of bad habits, insufficient practice, or impaired consciousness, swallow their sputum. In the majority of cases this difficulty can be remedied by teaching. For diagnostic purposes the total output of sputum for twenty-four hours is collected in a suitable receptacle, and should be free from admixture of antiseptics.

The sputum is examined in bulk with reference to its quantity, reaction, consistence, air-content, apparent composition, color, and odor.

THE AMOUNT.

The amount of sputum voided in twenty-four hours may be very great. On the other hand, even when every effort is made to expectorate, very little is produced. Some phthisical patients, in spite of violent coughing, raise very little, and that is of a very tenacious quality.

Scanty, or *absent,* sputum may be evidence of the first stage of bronchitis, asthma, laryngitis or pleurisy. While in children

under 6 or 7 years of age, it is usually absent because it is swallowed.

Abundant sputum is of importance in a general way because it denotes, in acute infectious conditions particularly, that nature is prompt to relieve the body of an abundant secretion, which, if retained, might cause serious consequences by further reducing the already diminished respiratory capacity. When large amounts of sputum are voided at short intervals, alternated with periods of practical absence, one may infer a tuberculous, gangrenous or bronchiectatic activity, or rupture into the lung of an abscess of the lung, liver, kidney or subphrenic space.

CONSISTENCE AND APPARENT COMPOSITION.

The consistence of sputum may bear a certain relation to the amount when, if abundant, the consistence is lessened, and *vice versâ*. This relation is by no means constant.

Ordinarily, sputum is "slimy." It may, however, be serous, purulent, or bloody. The peculiar slimy characteristic of sputum depends upon the amount of mucus contained, while its stickiness depends, in part, upon the mucin and, in part, upon the proteid content. This is especially marked in lobar pneumonia.

(a) *Watery, or serous, sputum,* which is frequently blood-tinged, occurs in pulmonary edema, and in catarrhal influenza. Gastric disorders in neurotic old people may give rise to a thin, watery expectoration of considerable quantity, which is partly regurgitated and partly hawked up.

(b) *Viscid, or sticky, sputum,* which adheres to the bottom of the container, even when completely inverted, is somewhat characteristic of lobar pneumonia, but may also be seen in phthisis, pertussis, and in broncho-pneumonia.

(c) *Mucous sputum*, a clear diffluent sputum resembling egg-albumin, composed chiefly of mucus, is observed in the early stage of pneumonia, bronchitis, and phthisis; at the termination of an asthmatic attack, in pertussis, pharyngitis, laryngitis, measles, and influenza.

(d) *Muco-purulent sputum* is composed of mucoid sputum in which occurs a varying number of streaks and masses of

opaque, yellow or greenish pus. It is noted toward the end of measles, pertussis, in resolving pneumonia, during phthisis, and in subacute and chronic bronchitis.

(e) *Purulent sputum,* which is composed purely of pus, is rather rare, and, when observed, indicates rupture of a liver, kidney or subphrenic abscess, or purulent pleurisy into the respiratory tract. Opaque yellow sputum, consisting largely of pus, is found in bronchiectasis, phthisical cavities, broncho-pneumonia, and in the chronic or later stages of acute bronchitis.

(f) *Numular sputum.* Ring or coin-shaped masses of sputum, which sink immediately in water, occur at times in bronchiectasis, chronic bronchitis or phthisical cavity.

(g) *Frothy sputum* may be observed in bronchitis, broncho-pneumonia, and emphysema. Its most important relation is in pulmonary edema, in which condition it is full of air and resembles frothy soap-water.

COLOR OF SPUTUM.

(a) *Rusty sputum,* due to evenly distributed altered blood, is generally indicative of lobar pneumonia, but may also be observed in tuberculosis pulmonalis.

(b) *Prune-juice expectoration,* a rather fluid expectoration discolored by altered blood, is seen in gangrene and in cancer of the lung.

(c) *Current-jelly sputum* is said to be characteristic of cancer of the lung.

(d) *Black sputum.* Sputum which is very dark or black streaked or specked, is found in persons who have inhaled coal dust or smoke for long periods of time. It is sometimes seen in gangrene of the lung.

(e) *Yellow, or green, sputum* may be caused by abscess of the liver which has ruptured into a bronchus (bile pigment), and also in some cases of pneumonia (altered blood), and in pulmonary infections with chromogenic bacteria.

(f) *Shreds and casts* may be observed in the sputum of chronic bronchitis, diphtheria, and rarely in pneumonia. Casts, unless large and branching (Fig. 4), are more apt to be found

during microscopic search. Suspicious particles should be floated in water against a black background, and teased out with needles.

(g) *Blood-streaked sputum.* Sputum streaked or discolored with blood, may be due to violent vomiting or coughing, diseased tonsils or leakage from an aortic aneurism. Under these conditions it will appear as light red, but slightly altered blood. It may be present as a sequel of hemoptysis or abscess

FIG. 4.—BRONCHIAL CAST, FROM CASE OF FIBRINOUS BRONCHITIS IN SERVICE OF DR. JUDSON DALAND (ORIGINAL).

of the lung in broncho-pneumonia, empyema or in bronchitis.

If the blood is dark or black it may be due to pulmonary infarction. Most commonly hemoptysis is observed in phthisis, when it recurs intermittently for days or weeks. Finally, it should be remembered that malingerers may simulate disease of the respiratory tract by sucking blood from wounds of the gums, lips, tongue or cheeks.

(h) *Hemorrhagic sputum* is observed in traumatic or tuberculous hemorrhage, in hemorrhagic infarctions, and in lobar

pneumonia. Also in tumors in or near the respiratory tract, and finally in congestion of the pulmonary circulation.

Certain derivatives from the blood-pigment produce in the sputum shades very similar to that of blood—the *rusty sputum* of pneumonia, for instance. The coloring matter here is partly changed blood and partly a yellowish-red derivative of blood-pigment, about which very little is known. Peculiar lemon-colored and grass-green shades are also frequently observed in pneumonic sputum. These likewise are due to changed blood-pigment. Such sputa respond to Gmellin's test for bile-pigment (see page 214). A peculiar light-brown sputum is sometimes observed in heart disease, particularly mitral, in which amorphous blood-pigment is found encapsulated in the alveolar epithelium (heart-failure cells). A type of green sputum has been observed in cases of lung tumor, the nature of the pigment in question is as yet unknown.

(i) *Extraneous discolorations.* Other noticeable discolorations of the sputum are observed from the admixture of inhaled dust-particles. The *black sputum* of miners, and the *blue sputum* of workers in ultramarine, are examples of this class.

Finally, it should be remembered that the sputum voided, following the ingestion of certain food-stuffs, may be discolored from contamination with these occurring in the mouth and pharynx. A *greenish* discoloration of the sputum is sometimes the result of the activity of certain chromogenic bacteria, especially the *Bacillus virescens*.[1] *Yellow* and *bluish* sputa of probable bacterial origin have occasionally been observed.

REACTION.

The reaction of fresh sputum is generally alkaline; it may occasionally be acid, and usually becomes so after standing for some time through decomposition resulting from bacterial growth.

AIR CONTENT.

Sputum is often more or less foamy or frothy, due to the presence of air. Other things being equal, the content of air in the sputum is greater the finer the bronchi from which the

[1] Frick: Virchow's Archiv., Vol. cxvi., 1889.

sputum is derived. The consistence of sputum often has a bearing on this. The amount of air contained can easily be determined by comparing its specific gravity to that of water. Air-containing sputum will float; airless sputum will sink.

THE ODOR.

Fresh sputum has a rather characteristic, but indescribable, odor. On standing it may acquire a disagreeably nauseating odor from decomposition, resulting from contained bacteria. Freshly voided sputum has a very decided odor in bronchiectasis, purulent bronchitis, tuberculosis, gangrene of the lung, and lung abscess. The disagreeable odor here arises from the activity of putrefactive bacteria. Stagnation of the secretion in cavities favors decomposition. In this way the foul odor of the expectoration in consumptives may be imparted to the breath. Finally, it must be remembered that fecal vomiting and diseased conditions of the mouth may be responsible for the odor of the breath and sputum, as will also the ingestion of certain volatile drugs, including alcohol.

HEMOPTYSIS.

True hemoptysis means the expectoration of an appreciable amount of pure, or nearly pure, blood, not merely sputum tinged with blood. The amount of blood may continue small, and may persist for many days or may, as in the case of rupture of an aortic aneurism, be sufficiently large to cause death in a short time.

Before making a diagnosis of hemoptysis it is necessary to exclude blood coming from the nose, pharynx, larynx, buccal cavity, and tonsils.

The Common Causes of Hemoptysis.—(a) *Pulmonary disease,* usually tuberculous. It may also occur in the early stages of lobar pneumonia, abscess, bronchiectasis, gangrene, and cancer of the lung.

(b) *Cardiac disease.* Here it may be the result of venous obstruction occurring in the course of valvular disease. This is a not uncommon cause of slight but long-continued bleeding.

(c) *Vascular disease.* The most important condition is

rupture of an aneurism into the respiratory tract. Leakage from the same cause may cause slight but persistent hemoptysis.

(d) *Diseases of the blood.* Hemoptysis may occur during the course of hemophilia, purpura, leukemia, scurvy, and severe anemia. It has been noted occasionally in the course of some of the exanthemata.

(e) *Miscellaneous causes.* Vicarious menstruation and hysteria.

GROSS EXAMINATION OF SPUTUM.

Examination should be made both upon a white and a black background. Many sputa appear to the naked eye to be homogeneous—pure mucus, pure pus, pure blood; but sometimes not only may the sputum of one patient vary, but differences may be noted in each expectoration.

"*Dittrich's plugs*" are yellowish-white masses, the size of a mustard-seed, and are easily seen over a black background. They come from the smaller bronchioles in putrid diseases, especially in putrid bronchitis and pulmonary gangrene. Microscopically they are composed of clumps of bacteria and fatty acid crystals. They have a very intense and disagreeable odor. Somewhat similar plugs may be encountered in the sputum in follicular tonsillitis. These plugs should not be confounded with those little masses described by, and known as, Curschmann's spirals. (See page 27.)

Formations consisting of *fibrin* are encountered in certain infections, and should be easily recognized by their white color, tenacious consistence, and sometimes by their shape (casts and molds).

Foreign bodies are sometimes aspirated into the respiratory tract, where they may remain for years without causing any symptoms until a fit of coughing dislodges them and they appear in the sputum.

Concretions are sometimes, though very rarely, formed in the lungs during chronic inflammatory conditions. These are accidentally coughed up when they may be found in the sputum. Occasionally these stones may have their origin in the crypts of the tonsils, or they may be calcified lymph nodes that have ulcerated into the lung.

MICROSCOPIC EXAMINATION OF THE SPUTUM.

A microscopic examination of the sputum reveals the presence of cells, elastic fibers, casts, spirals, crystals, and microorganisms. It is advisable to examine first the fresh unstained sputum, as the presence of fungi or crystals in the sputum, and the nature of many of the cellular elements, can be determined only in this way. Afterward dried and stained cover-glass preparations may be made for more minute and detailed study.

Preliminary Examination.—This is necessary in order to locate suspicious particles which may be scattered throughout the large mass of sputum. This is made either with the unaided eye or with a hand-lens. A thin layer of sputum is necessary to successful examination. For this purpose a moderate-size Petri dish and cover is much better than the flat pieces of glass ordinarily employed, which are uncleanly and difficult to handle. A small amount of sputum is placed on the inside of the cover, and the other half of the dish pressed down into this, the rim very successfully preventing the escape of excess of material.

The Unstained Specimen.—For the purpose of isolating any characteristic particles, the sputum should be spread out in a thin layer in the dish, and the material teased out with needles or tooth-picks. Having located a likely particle, it is transferred to a clean slide and flattened out by pressing a cover-glass down upon it. This should be examined first by the low, and later by the medium, power objective.

Appearance.—Most sputum consists, microscopically, of a ground-work of *mucous matrix* of indefinite structure and appearance, in which are imbedded a variety of microscopic objects, principally cells.

1. *Pus cells.* The number of these indicates, in a general way, the purulent nature of the specimen. The character of the corpuscles varies greatly. Their size is from 7 to 10 micromillimeters, they appear more or less granular, are sometimes distinctly pigmented, containing one or more irregular nuclei. The granules are composed some of proteid, some of fat, and some of extraneous *débris.*

2. *Epithelial cells* found in the sputum differ from the pus cells, being usually larger in size, and by exhibiting one rather

large vesicular nucleus. Various types of epithelia are met in the sputum, and their recognition is of considerable value in locating the origin of the expectoration, although, many times, the conditions to which they have been subjected after being shed have so altered their appearance that little knowledge can be gained from their study. *(a)* Squamous epithelia are derived from the mouth, the pharynx, and from part of the larynx. *(b)* The cylindrical epithelium is derived from the nose or from the smaller bronchi, and are seen as pear-shaped or oval cells, some of which possess cilia. *(c)* Pulmonary, or alveolar epithelium, is oval and measures from 20 to 30 micromillimeters in diameter.

3. *"Heart-failure" cells.* These are oval or round, pigmented, alveolar cells. When numerous their presence is said to be indicative of chronic passive congestion of the lungs, usually depending on the failing compensation of cardiac valvular disease. Their presence is, therefore, usually associated with the common signs in the lungs, which are indicative of failing compensation, viz.: moist râles, mucous expectoration, and cyanosis.

4. *Eosinophiles* may occasionally be found in large numbers associated with Charcot-Leyden crystals in the expectoration of bronchial asthma.

5. *Red-blood cells.* The appearance of red-blood cells in the sputum will depend largely upon the length of time that they have been shed. As they grow old they become pale, shadowy, and fragmented. The finding of a few red-blood cells in the sputum is of no diagnostic import. They occur naturally in large numbers in hemoptysis, and are constant and more or less abundant in all inflammatory diseases of the lungs, particularly phthisis.

6. *Casts.* These may vary in size from those which represent molds of the trachea and larger bronchi to those coming from the smaller bronchioles, and which are from ¼ to 1½ inches long. These smaller casts are the more common, and when present usually require the use of the low-power objective to demonstrate them. These casts usually occur in one of the three following diseases: the largest in diphtheria, medium-size in fibrinous bronchitis, and the smallest in lobar pneumonia.

7. *Curschmann's spirals* consist of worm-like spirals 1 to 2 centimeters long, and about 1 millimeter wide. They are

more or less opaque, and are usually found surrounded by a thick, clear mass of mucus. They frequently show a central, undulating, thread-like core around which are twisted, in a spiral manner, the mucous threads. Entangled in these spirals are usually eosinophiles and Charcot-Leyden crystals. They occur frequently in the sputum of bronchial asthma, more rarely in phthisis, bronchitis, and in lobar pneumonia. Their presence may be of service in differentiating bronchial from other forms of asthma.

Crystals.—(a) These are found usually only when the sputum has been retained within the body for a length of time. *Crystals of fat or of fatty acids* are most frequently encountered. They appear as long, slender needles, either singly or grouped into fine rosettes or sheaves. They are readily soluble in potassium hydrate or in ether. This solubility is easily determined by allowing a little of either fluid to flow under the edge of the cover-glass while observing the crystals in question.

(b) *Crystals of calcium phosphate* may be encountered under conditions of retention and stagnation.

(c) *Charcot-Leyden crystals* are occasionally encountered, particularly in the expectoration of bronchial asthma, and are here accompanied by eosinophiles. They appear as colorless, elongated double pyramids, varying considerably in size. They are often so small that high magnification is required to reveal them.

(d) *Cholesterin crystals* are but rarely seen in the sputum. They occur as transparent, colorless, rhomboidal platelets, with notched or irregular angles and ends.

(e) *Hematoïdin crystals* are derived from hemoglobin by a process of decomposition, and occur as needles and rhomboidal platelets of reddish and brownish hue. They are found chiefly in the sputum from old abscesses or perforating empyemas.

(f) *Leucin globules and tyrosin crystals* are found in putrid sputum from old perforating abscesses or in putrid bronchitis.

(g) *Calcium oxalate,* in minute octahedral crystals, are occasionally met.

Elastic Fibers.—When the lung-tissue is destroyed to any extent by pathologic processes, elastic fibers are apt to be

encountered in the sputum. Their presence in the sputum proves conclusively the occurrence of some destructive process within the lung. Hence their importance in the diagnosis of tuberculosis of the lung before the appearance of tubercle bacilli. Elastic fibers occur also in pulmonary abscess and gangrene.

These fibers are usually detected in a thin layer of sputum examined microscopically. In this examination care must be observed to avoid confusing true elastic fibers with the somewhat similar vegetable fibers, which latter are generally larger and less uniformly wavy.

To detect particles or shreds of elastic tissue in the sputum, suspicious lumps should be thoroughly mixed with an equal quantity of 20-per-cent. sodium hydrate solution; then a large volume of water is added, and the whole allowed to sediment for a few hours. The sediment is then removed and examined under the microscope for the characteristic fibrillated masses.

If elastic tissue is not found by this procedure, the entire quantity of the twenty-four-hour specimen should be boiled with an equal quantity of the sodium hydrate solution. The resulting gelatinous mass is then mixed with several volumes of water, and allowed to sediment. About 15 cubic centimeters of the sediment is now removed with a pipette and centrifuged for fifteen minutes. The final precipitate is now carefully removed and examined as above.

Fragments of tumors are occasionally encountered in the examination of sputum. These should be removed and prepared for sectioning and staining.

PREPARATION OF THE STAINED SPECIMEN.

Another suspicious particle having been isolated and removed from the mass of sputum, it is transferred to and carefully spread upon a clean cover-glass. This should then be treated to fixation and staining, the technic of which will depend upon the nature of the information sought. (For methods of staining and for differentiation of the organisms, see section devoted to Bacteriologic Methods, page 244.)

PULMONARY ACTINOMYCOSIS.

This condition, also known as disease of the ray-fungus, occasionally causes disease of the lung, but is exceedingly rare in this country. The characteristic yellowish- or grayish-green granules, if found, are often sufficient for a diagnosis which should, however, always be confirmed by microscopic search. In some cases the characteristic microscopic rosettes with clubbed rays are found; in others only branching threads, staining by Gram's method, will be found.

Clinically, the course of the disease is similar to that of pulmonary tuberculosis, except that instead of the tubercle bacillus, the ray-fungus is found.

ECHINOCOCCUS.

Rarely echinococcus hooklets enter the pulmonary tract and appear in the sputum. They usually originate in abscesses of adjacent organs, particularly the liver. (See section, "Animal Parasites," page 101.)

DISTOMUM PULMONALE.

This is a disease of the lungs, usually occurring in Asia, but occasionally encountered in other parts of the world. It is due to the presence of a worm which, by causing hemorrhage, may be mistaken for phthisis. When present its eggs can always be demonstrated in the sputum. They are oval, of a brownish-red color, 0.08 millimeter long, and 0.056 millimeter broad, surrounded by a cuticle. (See section, "Animal Parasites," page 101.)

III.

THE BLOOD.

THE CHEMICAL COMPOSITION OF THE BLOOD.

A GENERAL idea of the composition of the blood may be had from the following table, which is taken from Simon's "Physiologic Chemistry." The calculations are made for 1000 parts by weight.

Corpuscles	480.00	parts
Water	276.90	"
Oxyhemoglobin	193.90	"
Stroma, including salts	9.20	"
Plasma	520.00	parts
Water	477.37	"
Albumins	35.88	"
Extractives	2.39	"
Inorganic salts	4.36	"

The predominating solid substance in the blood is oxyhemoglobin; it represents 10 per cent. of the total weight of the blood, 40 per cent. of the weight of the corpuscles, and 65 per cent. of all organic matter present.

The mineral constituents comprise sodium, potassium, calcium, magnesium, and iron.

Fats are present to the extent of from 0.2 to 0.3 per cent. These may be temporarily increased after the ingestion of much fatty food, and also in many pathologic conditions.

The plasma normally contains small amounts of oxygen and nitrogen in solution, with varying amounts of carbon dioxide.

METHODS OF OBTAINING BLOOD FOR EXAMINATION.

The tip of the finger or lobe of the ear are the sites usually selected from which to obtain specimens. In the majority of

examinations only a small amount—a few drops—is necessary. This is obtained by simple puncture of the skin made with a glover's needle, the half-point of a new steel pen, a *Daland* lancet (see Fig. 5), or the so-called pistol-knife. The two last-mentioned instruments are to be preferred because they permit of regulation of the depth of the puncture.

For larger quantities of blood, a few cubic centimeters or more, it is advisable, provided there is no contraindication, to obtain the specimen by the use of wet cups. It must be borne

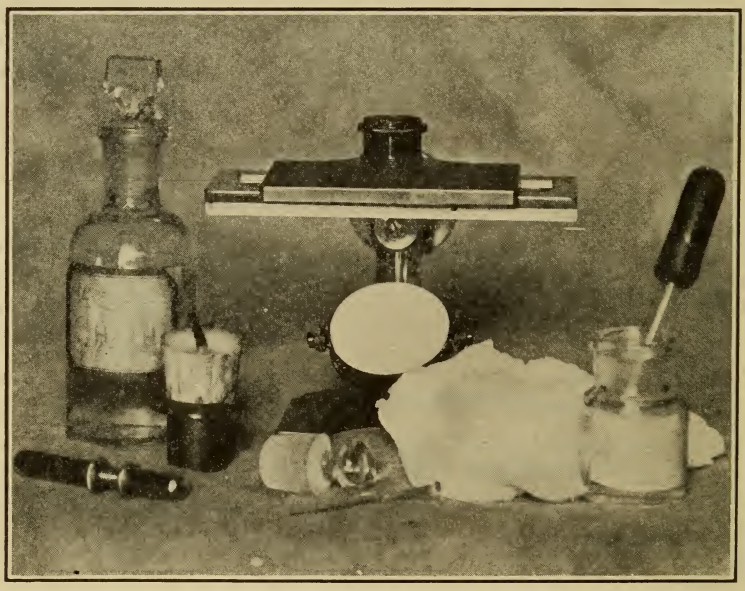

FIG. 5.—FLEISCHL HEMOGLOBINOMETER, SURROUNDED BY ACCESSORIES NECESSARY TO PERFORMANCE OF THE TEST, INCLUDING DALAND LANCET.

in mind that by this method there is always more or less admixture of lymph. Another method is to draw the blood from a dilated vein into a large sterile antitoxin syringe. Lastly, an ordinary venesection may be resorted to.

The withdrawal of blood, if aseptically performed, is practically free from danger and need disturb the patient very little. Before proceeding in any case it is advisable to determine the absence of hemophilia in the patient.

Appearance of Fresh Blood.—The exuding drop of blood

shows even to the naked eye a number of properties. The redder it is, the richer it is in oxyhemoglobin; the darker, the greater the amount of reduced hemoglobin. Microscopically, it reveals a great number of cellular elements; these are colored and colorless discs.

The *red cells* appear as non-nucleated bi-concave discs, measuring on the average 7 micro-millimeters in diameter. Viewed singly through the microscope by transmitted light, they are of pale-greenish hue. The *colorless cells* or *white corpuscles* are, as a rule, somewhat larger than the red cells, and present either mono- or poly-nucleated protoplasm.

The plaques, or *blood-platelets,* appear as minute, colorless discs measuring less than half the diameter of the red cells. They usually occur in groups or bunches of half a dozen or more, and are present in normal blood to the number of about 635,000 per cubic millimeter.

There are no other morphologic constituents of the blood.

Color.—The color of normal blood is due to the presence of an albuminous substance in the corpuscles termed hemoglobin. In the arterial blood it is in combination with oxygen, and is here termed oxy-hemoglobin. In the venous blood a mixture of both hemoglobin and oxy-hemoglobin occurs. With a preponderance of oxy-hemoglobin, the blood tends to a scarlet hue; when the hemoglobin predominates, the blood is of a bluish color.

Pathologic Changes in Color.—In coal-gas poisoning the blood is cherry-red. After poisoning from potassium chlorate, aniline, hydrocyanic acid, and nitro-benzol, the blood is brownish-red or chocolate color. In extreme cases of leukemia the blood may have a milky appearance due to the excessive number of white blood cells present.

The Odor.—This is characteristic and differs in different species of animals. It is due chiefly to the presence of volatile fatty acids.

The Taste.—The taste of blood is salty, but at the same time insipid.

THE SPECIFIC GRAVITY.

The specific gravity seems to vary with the amount of hemoglobin. It is influenced by the age and sex of the indi-

vidual, the process of digestion, exercise, pregnancy, etc. The normal average in adults varies between 1.058 and 1.062.

Determination of the Specific Gravity (method of Hammerschlag).—A cylinder about 10 centimeters in height is partly filled with a mixture of benzol (sp. gr. 0.889) and chloroform (sp. gr. 1.526), so that the specific gravity of the mixture lies between 1.050 and 1.060. Into this a drop of blood is allowed to fall directly from the finger. It is then brought into suspension by the addition of either a little chloroform or benzol, according to the tendency of the drop to sink or rise in the cylinder.

As soon as the drop remains stationary in the fluid the specific gravity of this is taken by an accurate hydrometer (one reading to the fourth decimal should be used). The reading represents the specific gravity of the blood tested.

THE AMOUNT.

The total amount of blood in the normal adult is said to amount to about one-twelfth or one-fourteenth of the body weight.

THE REACTION.

The reaction of the blood is slightly alkaline, due to the presence of the mono-sodium carbonate and the di-sodium phosphate in solution in it. The reaction may be roughly determined by drawing a strip of neutral litmus paper, which has been thoroughly moistened with a concentrated solution of common salt, through the blood, and then rapidly washing the corpuscles off with the same solution. Owing to the development of certain acids, the alkalinity of the blood rapidly diminishes after it is shed. This fact renders this determination a rather difficult matter. The normal variation of alkalinity is very slight. By accurate titration the normal degree of alkalinity of the blood, under normal conditions, corresponds to from 325 to 360 milligrams of sodium hydrate for every 100 cubic centimeters of blood.

QUANTITATIVE CLINICAL METHODS.

The usual clinical methods applied to the blood in the study of disease are: the estimation of the percentage of hemoglobin, the enumeration of the erythrocytes and the white blood-corpuscles, and a differential count of the various white elements.

The apparatus necessary for the performance of these several examinations are as follows:—

I.—A good microscope with 3, 6, and oil-immersion ($\frac{1}{12}$) objectives.

II.—A hemoglobinometer (Gower's, Fleischl's or Sahli's).

III.—A Thoma-Zeiss hemocytometer.

IV.—Slides, covers, stains, etc. (For complete list see Appendix.)

Estimation of the Percentage of Hemoglobin.—Method of Gower: The tip of the finger or lobe of the ear is punctured after having been thoroughly cleansed with alcohol, followed by ether. The first drop obtained is wiped away, and the second, which should flow without assistance either by pressure or rubbing, is drawn up by suction into the pipette to the 20 centimeter mark; all adhering blood is wiped from the outside of the tube. The contents are now immediately forced out into the graduated observation tube, which has previously had a few drops of distilled water placed in it (this is to prevent the blood from coagulating on its walls). Be sure that all blood contained in the mixing tube has been washed into the graduated tube. Now, while holding the two tubes side by side directly against the light, add distilled water, drop by drop, until the shade of color is the same in the two tubes. The division on the scale to which the fluid rises will express the per cent. of hemoglobin.

Method of Fleisch.—The instrument (Fig. 5) consists of a metal stand having a horizontal table with a circular aperture in its center, beneath which is placed a reflector of plaster-of-Paris. Immediately beneath the aperture and above the reflector, a graduated wedge of tinted glass is arranged to move in a horizontal plane by means of a milled thumb-screw. This graduated wedge of glass is shaded to represent varying degrees

of hemoglobin content, and carries with it a scale indicating the blood-strengths. For collecting and diluting the blood for examination, a pipette and diluting chamber are furnished. *The technic* is as follows:—

By the aid of the pipette which is furnished with the apparatus, an exactly determined amount of blood is dissolved in a measured quantity of distilled water (the contents of one-half of the diluting chamber, the other half being filled with an equal amount of distilled water). The cell thus prepared is then placed over the aperture in the table, and artificial (candle, lamp or gas) light is directed through it by means of the reflector. The part of the wedge is now searched for, which accurately compares in tint with the solution of blood under

Fig. 6.—Thoma-Zeiss Hemocytometer in Case. (A. H. Thomas.)

examination, and the number of the scale is read off that corresponds to this point on the glass wedge. It is a matter of common experience that the grading of these instruments is too high, and that a specimen of blood that corresponds to 90 or 95 on the scale is normal.

Enumeration of the Corpuscles. — The Thoma-Zeiss apparatus consists of a counting slide and two diluting pipettes, termed "melangeurs" (Fig. 6). This slide is so constructed that it contains a chamber in its center having a depth of $\frac{1}{10}$ millimeter. The center of the floor of the cell is divided by fine microscopic lines into minute squares, the sides of which are equal to $\frac{1}{20}$ millimeter. The cell is completed by the application of a special cover-glass. Under these conditions each cube as outlined has a capacity of $\frac{1}{4000}$ cubic millimeters.

These small squares are divided into groups of sixteen by means of double ruled lines, each group of sixteen small squares constituting a large square. There are sixteen of these large squares in the slide. There are, therefore, two hundred and fifty-six small squares in the sixteen large squares. If we include also the small squares forming the boundaries between the large squares, the total number of small squares will be four hundred (Fig. 9). This counting chamber is used in estimating both the red and the white cells, also the number of cells in the cerebrospinal fluid, pleural effusions, etc.

Estimation of the Erythrocytes.—The "melangeur" having the smallest bore and having marks at 0.5, 1.0, and 101, is used in this estimation. The drop of blood issuing from the puncture is drawn up to the 0.5 mark, the tip then quickly freed from adherent blood, and immersed in a 2½-per-cent. solution of potassium bichromate (or Hayem's solution, for which see Appendix), which is drawn up to the 101 mark. The tip of the tube is now stopped with the finger and the tube vigorously shaken, for at least a minute, to insure thorough and even dilution of the blood. The portion of the diluting fluid contained in the capillary part of the tube is blown out and wiped away, and the next drop of the mixture placed in the center of the floor of the counting chamber. This drop should be entirely free from bubbles. The cover-glass is now applied over this and pressed down firmly around the edges until Newton's rings appear.[1] A few moments should now pass before counting to allow the corpuscles to settle to the bottom of the chamber.

A simple and practical method of arriving at the number of red corpuscles obtained in a cubic millimeter of blood is the following: Select and count from the various parts of the chamber five large squares (Fig. 7), counting the border cells only upon the upper and right-hand lines. The total of small squares counted will be eighty. To the total of the corpuscles counted in the five large squares add four 0000, and the number resulting will be the number of red corpuscles in 1 cubic millimeter of undiluted blood.

[1] A series of curved, colored lines appearing behind the plane glass surfaces, where they are firmly pressed together.

ENUMERATION OF THE RED CELLS.—*Explanatory note:* $\frac{1}{4000}$ cc. equals the cubic capacity of one small square. $\frac{1}{200}$ equals the dilution of the specimen of blood. Five large or eighty small squares is the number of squares counted. Then the number of cells per cubic millimeter in the undiluted specimen will equal the number of cells in one small square multiplied by the dilution times 4000, viz:—Let x equal cells per cubic millimeter, and let y equal number of red blood-cells in eighty small squares. Then, $\frac{y}{80} \times 200 \times 400 = x$ which simplified is the same as $y \times 10000 = x =$ the number of red blood-cells in one cubic millimeter of undiluted blood.

A Second Method.—Count all the small squares (400); divide the number of cells counted by the number of squares. This will give the average number of cells in one square ($\frac{1}{4000}$ cubic millimeter) of diluted blood. To determine the number

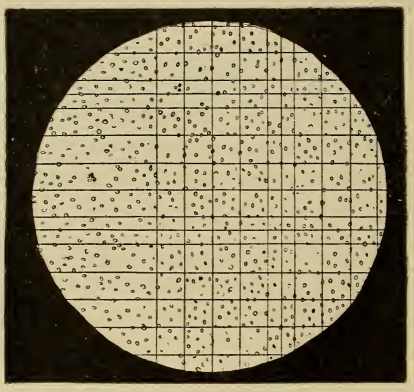

FIG. 7.—APPEARANCE OF FIELD OF THOMA-ZEISS HEMOCYTOMETER WHEN PROPERLY MOUNTED FOR COUNTING THE RED CORPUSCLES.

of cells in 1 cubic millimeter of undiluted blood, it is only necessary to multiply this by 4000 and this product by the dilution.

EXAMPLE.—Suppose 1200 cells have been counted in 400 squares, then the average in one small square is three; this, multiplied by the number 4000 will equal 12,000, the number of corpuscles contained in 1 cubic millimeter of diluted blood, since the dilution was 200 times; then 12,000 x 200 will equal 2,400,000 red blood-cells in 1 cubic millimeter of undiluted blood.

Recently Drs. Einhorn and Laporte have perfected a rapid method of counting by means of a specially constructed counting diaphragm.

The normal number of erythrocytes is usually placed at 5,000,000, although it is not uncommon to find the normal in many patients maintained above this figure.

Determination of the Erythrocytes by the Centrifuge.—The Daland hematokrit (Fig. 8) offers a quick, simple, and accurate method of determining the number of red corpuscles in the blood. The two great advantages of this process are, first the elimination of the personal equation, and second, as there is no dilution of the blood required, the frequent source of error arising from this cause does not enter in.

The hematokrit consists of an extremely light, though very strong, metal frame containing two glass tubes, each 50 millimeters long and graduated in one hundred parts, and having a uniform lumen of 0.5 millimeter.

The length of the percentage tubes and their distance from the center of the revolving hematokrit frame must always be

FIG. 8.—DALAND HEMATOKRIT, SHOWING ONE PERCENTAGE TUBE IN POSITION. (A. H. THOMAS.)

the same as was used in the original experiments by which the comparative determinations were made, since any variations in these factors will produce corresponding variations in the results obtained.

THE TECHNIC.—The blood having been secured as outlined on page 31, the blunt end of the graduated percentage tube is attached to a suction pipette and the tube completely filled with freshly drawn blood. After any excess of blood has been carefully wiped away, the blunt end of the tube is placed in the distal cap of the hematokrit frame, and the other end pressed down upon the inclined plane of the proximal cap until it falls into and locks in the cavity. The second tube should be similarly treated and placed in the opposite side to serve as a check. It is absolutely necessary that the entire procedure should be performed with celerity so as to anticipate coagulation. After filling, immediately place the frame upon the spindle of the high speed centrifuge, and turn the handle at the uni-

form rate of eighty turns per minute for exactly two minutes. This will produce 10,000 revolutions per minute in the spindle. At the expiration of this time all the corpuscles will be found to occupy the distal end of the tube, a thin, almost invisible, whitish line of white corpuscles appears between them and the plasma, which will be found to occupy the proximal portion of the tube. With the aid of a hand lens the height of the column of red corpuscles is read off, and the reading multiplied by 100,000, which converts the volume percentage into the number of corpuscles per cubic millimeter of the blood under examination.

The speed of the handle of the centrifuge must be uniform throughout the whole period, because this rate of rotation is the measure of a definite amount of centrifugal force generated in the revolving arms of the hematokrit frame. If the rate of rotation is either increased or decreased, the height of the column of cells will be similarly affected, thereby producing either too low or too high a reading.

A possible source of uncontrollable error is the variation in size of the individual erythrocytes which occurs in certain pathologic states. The most accurate results by this method will therefore be obtained when the corpuscles are of uniform size. This fact has been taken advantage of in the estimation of the volumetric quotient, and its relation to diagnosis and prognosis in certain blood-conditions (see page 41).

The Percentage of Erythrocytes.—To obtain the percentage of red blood-corpuscles, take the first two left-hand figures of the count in 1 cubic millimeter and multiply these by two; this will represent, approximately, the percentage of red blood-cells.

Explanation.—If we consider normal blood to contain exactly 5,000,000 corpuscles, then the figure 50 x 2 would equal 100 per cent., or normal. If, however, only 4,790,000 were counted in 1 cubic millimeter of blood, then 47 x 2 will equal 94 per cent. of erythrocytes.

The Color-Index.—From a knowledge of the percentage of hemoglobin and the percentage of cells, we are in a position to calculate the average color-index per cell, *i.e.*, its relative hemoglobin-content as compared with the normal cell.

Example.—If the percentage of hemoglobin be 100 and

the percentage of cells 100, then the color-index of each cell would be 1.0 or normal, viz.:—

$$\frac{100\ \%\ \text{hemoglobin}}{100\ \%\ \text{red cells}} = \text{color-index } 1.0.$$

If the percentage of hemoglobin be 67, and the percentage of corpuscles 90, then each individual cell will contain less than a normal amount of hemoglobin:—

$$\frac{67\ \%\ \text{hemoglobin}}{90\ \%\ \text{red cells}} = \text{color-index } 0.74.$$

The Volumetric Quotient or the Volume-Index of the Erythrocytes.—The investigations of Capps[2] have demonstrated that the normal volume obtained by the centrifuge hematokrit is 50 per cent. in the normal specimen. This volume he designates as 1. The volume in pathologic alterations can be calculated in percentage of the normal volume, just as in the amount of hemoglobin estimated under similar circumstances. Having determined the volume by the hematokrit the erythrocytes are next counted and their number expressed in percentage by comparing with the normal. By dividing the volume of erythrocytes by the number of erythrocytes in the same blood (both expressed in percentage), we obtain the "volume-index" or "volume-value" of the erythrocytes, which is analogous to the color-index or hemoglobin as obtained above. This number in a measure of the average volume of the individual erythrocyte, and obviously under normal conditions, equals 1. Capps found that an increase in the volume-index of the erythrocytes is one of the most constant and accurate characteristics of pernicious anemia, which agrees with the well-known fact that many macrocytes appear in the blood in this disease, and that the color-index is also greater than one. In contrast to this, the so-called secondary enemas usually show a diminished volume-index; the same is true of chlorosis, in which affection the volume-index may be taken into account in determining the prognosis, since a normal or only slightly decreased volume-index gives a favorable prognosis, whereas a markedly diminished volume-index may be considered a bad prognostic sign.

[2] Jour. Med. Research, Vol. x 3, Boston, 1903.

When utilized in this way the volume-index may become a more reliable sign in chlorosis than is the percentage of hemoglobin or the color-index[3]

Estimation of the Leukocytes.—The Thoma-Zeiss apparatus is used for this estimation. The "white" pipette is of larger calibre than the "red" pipette, and makes a lower dilution (1 to 10 or 1 to 20). For diluting it is customary to use a 0.3- or 0.5-per-cent. solution of acetic acid. This solution preserves the white cells, at the same time decolorizing the erythrocytes, thus facilitating enumeration.

For mixing and preparing the slide the same method is

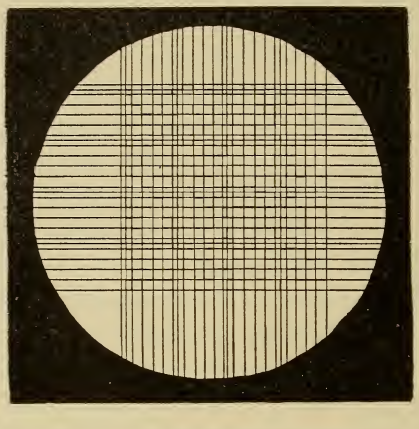

Fig. 9.—Ruling of Chamber of Thoma-Zeiss Hemocytometer.

employed as described for the red cells. It is customary to count the total number of squares (400) (Fig. 9), and to multiply the total number of cells counted by two hundred; the result will be the total number of white cells per cubic millimeter of blood.

Enumeration of the Leukocytes.—*Explanatory note:* $\frac{1}{4000}$ cc. equals the cubic capacity of one small square. $\frac{1}{20}$ equals the dilution of the blood, and 400 (all) the small squares counted. Then the number of leukocytes per cubic millimeter of undiluted blood will be found by dividing the number of cells counted by the number of squares counted and multiplying by the dilution times 4000 viz:—Let x equal the number of leukocytes per cubic millimeter, and y equal the number of cells counted in 400 small squares. Then, $\frac{y}{400} \times 20 \times 4000 = x$ or, $y \times 200 = x =$ leukocytes per cubic millimeter of undiluted blood.

[3] Sahli's Diagnosis quoting Capps; *loc cit.*

Examination of a Drop of Fresh Blood.—If a drop of fresh blood is placed upon a slide and a perfectly clean cover-glass allowed to fall upon this, a fresh preparation for examination will be produced, which may either be examined immediately or if sealed around the edge with a little vaseline, may be carried to the laboratory and examined within an hour. With the aid of a 6 objective many interesting points may be observed. First, the shape of the *red cells* and their hemoglobin staining, also rouleaux formation. Second, the structure of the *white corpuscles* by which the different varieties may be roughly differentiated. Third, the *blood-platelets*. Fourth, a rough idea of the relative number of red and white cells may be formed. This matter will be discussed more in detail a little later.

Studies of the minute structure of the leukocytes cannot be made satisfactorily by this simple method, as some of the forms are present only in small numbers, and are detected with difficulty. Further, prolonged examination of the fresh specimen allows time for changes, which rapidly obscure the identity of the cells; hence we are obliged to resort to a method of preparing a specimen which shall be permanent and which can be stained as a further aid to differentiation. These methods have the added advantage that they allow the investigator to work quite independently of the presence of the patient, to choose the time and place of examination, and permit at any time of verification and demonstration of the original result obtained.

Preparation of the Specimen.—The blood is obtained from the tip of the finger or the lobe of the ear after the manner described above. Cover-glasses should previously be prepared for reception of the fresh blood as soon as it is drawn. The cover-glasses should be square, of seven-eighths or one inch in diameter, and of special thinness (0.1 to 0.08 mm.). They should be washed carefully with warm water and soap, dried with fat-free gauze, and finally wiped off with ether. This is necessary to insure proper spreading of the blood between the two glass surfaces, which would be prevented by traces of fat or particles of fiber or dust, etc. Just before the glasses are used they should be wiped off with a piece of silk or tissue paper, and thereafter handled with care by the corners, or,

better still, with forceps. This preparation of the cover-glasses may be done in the laboratory or carried on at the bedside, as desired.

The glasses being ready and the finger cleansed and punctured, a small drop of blood is allowed to exude without pressure. Now a cover-glass is taken up diagonally between the thumb and finger of one hand, and its center allowed to touch the drop of blood. Immediately taking another cover-glass in the same manner in the other hand, it is allowed to fall upon the drop. If the glasses are perfectly clean and the maneuver properly executed, the blood will immediately spread out in a uniform layer between the two glasses. The two cover-glasses are now separated one from the other by a rapid sliding motion, in the strict plane of their surfaces, without the slightest lifting or tilting.

If the drop taken was small enough and the technic properly carried out in every detail, the result will be two uniform smears, covering the larger portion of the surfaces of the cover-glasses which, examined under the microscope, will present a uniform layer of corpuscles, with little if any overlapping of the individual cells.

Usually six films are prepared in this manner and allowed to dry in the air without heat.

Methods of Fixation.—In using the Romanowski stains no previous fixation of the albuminous film is required. Before applying any of the other stains it is necessary to "fix" the blood-film. If this is not done the corpuscles will be washed from the glass by the fluids applied.

1. A strip of sheet copper eighteen inches long and two or three inches wide, is placed upon a stand, and the flame from a Bunsen burner placed under one end. After allowing sufficient time for the plate to attain a maximum heat the films are placed upon it at a point where a drop of cold water fails to roll off but adheres to the hot metal and steams away. At this point fixation is complete in from one-half to three-quarters of an hour.

2. Fixation may be accomplished by immersion for five to ten minutes in a mixture of 1 part formaldehyde solution and 99 parts absolute alcohol.

3. For routine work when fair accuracy, accompanied by speed in obtaining results, is necessary, fixation in the naked flame of a Bunsen burner may be practiced. This is accomplished by holding the dried smear diagonally between the thumb and forefinger of one hand, film side up, and passing it through the flame a number (five to ten) of times with sufficient rapidity to prevent the fingers being burned.

METHODS OF STAINING: *Eosin and Methylene-Blue.*—The fixed film held in the cover-glass forceps is flooded with a ½-per-cent. alcoholic solution of eosin, which is gently washed off with distilled water after the lapse of one or two minutes. The counter-stain of methylene-blue, 1-per-cent. aqueous solution, is then added and allowed to act for from two to five minutes, according to the density of the film. Finally, washing in distilled water, blotting and drying, complete the process prior to mounting and examination.

This is an extremely simple, yet effective and permanent, method of staining blood-smears. Little difficulty will be experienced in obtaining satisfactory specimens. The eosin should not be allowed to act too long, but there is little danger of over-staining with the methylene-blue. Both of these stains are permanent when prepared, and can be kept until exhausted.

Properly performed this method gives very vivid and contrasting pictures, the nuclei of the different white cells taking various shades of blue, pale in the lymphocytes and dark in the polymorphonuclears. The protoplasm appears faintly tinted blue (paler than the nuclei). The eosinophilic granules are a bright pink. The erythrocytes a dusky red.

Ehrlich's Triacid Staining Method (for formula and prepation see Appendix).—The smear is taken in the cover-glass forceps and a few drops of the prepared stain placed upon it and allowed to remain for about five minutes. There is little danger of over-staining. The specimen is next washed in distilled water and thoroughly dried prior to mounting. If mounted in Canada balsam it is imperative that this should not contain any chloroform, otherwise the colors will gradually become blurred.

The Romanowski or "Universal" Staining Method.—Leishmann's modification of the Romanowski stain, as made by

Wright,[4] is the stain used. This method is altogether quicker and easier than the method of Ehrlich. It requires no fixing fluid or heating apparatus, and gives pictures which are uniformly superior.

Technic.—Allow three or four drops of the prepared stain to fall upon the smear and permit it to remain one-half minute, rocking the cover gently so as to insure an even distribution of the stain. No attempt is made to check evaporation. At the end of one-half minute add double the quantity of distilled water, *i.e.,* six to eight drops, and allow it to mix with the alcoholic stain. Immediate mixing is hastened by gently rocking the cover-glass. The film is now allowed to stain for five minutes. In thick smears ten minutes may be necessary. The stain is now gently washed off with distilled water, and a few drops of water are allowed to rest on the film for one minute, when it should be dried in air and mounted.

Appearance of Blood-Films.[5]—Erythrocytes, pale pink or greenish semi-transparent.

Polymorphonuclears.—Nuclear network, stained a very ruby-red color, or with sharply defined margins. Extra-nuclear protoplasm, colorless. Fine eosinophilic granules, red.

Mononuclears.—Nuclei ruby-red with extremely sharp, clear outlines. Extra-nuclear protoplasm, pale eau-de-nid or blue, occasionally showing a few red granules.

Lymphocytes.—Same as mononuclears, except that as a rule the nuclei are more deeply stained.

Coarse-Grained Eosinophiles.—Nucleus only, red, but not so densely stained. Granules pale pink.

Basophiles.—Granules very densely stained of a very purplish-black tint. Nucleus red, but usually more or less meshed by granules overlaying it.

Nucleated Red Cells.—Nucleus almost black, with sharp outline, extra nuclear portion gray.

Blood Plates.—Deep ruby-red with spiky margins, frequently showing a pale blue spherical zone surrounding the red center.

Bacilli and Micrococci.—Speaking generally, these stain

[4] Wright: Jour. Med. Research, Vol. vii, 1902.

[5] British Medical Journal, Vol. ii, page 757, 1901.

evenly blue, but by prolonging the staining period and subsequently decolorizing with absolute alcohol, many interesting variations may be noted with different organisms by which structural details are brought out not generally observed by other staining methods.

Malarial Parasites.—The body of the parasite stains blue and its chromatin ruby-red. In the case of the tertian parasite, Schuffner's dots are well marked in the containing red blood-corpuscles.

The only weak points in the Romanowski stain are said to be the deceptive resemblance between megaloblasts, certain lymphocytes and certain myelocytes, and failure to differentiate the basophiles.

TERMS IN COMMON USE IN CLINICAL HEMATOLOGY.

Anemia.—A condition of the blood in which there is a deficiency in one or more of the normal constituents.

Anhydremia.—A deficiency in the normal fluid of the blood.

Basophilic Granulation.—A peculiar granular degeneration of the red blood-cells which is noted in chronic lead-poisoning.

Hydremia.—An excess of fluid in the blood.

Leukocytosis.—An increase above the normal number of white blood-cells.

Leukopenia.—A diminution in the number of white blood-cells.

Lipemia.—The presence of an abnormal amount of free fat in the blood.

Macroblasts.—Nucleated red blood-cells of more than normal diameter.

Macrocytosis.—The occurrence of numbers of abnormally large red blood-cells.

Melanemia.—The presence of free pigment in the blood.

Microblasts.—Nucleated red blood-cells of abnormally small size.

Microcytosis.—The occurrence of numbers of abnormally small, red blood-cells.

Normoblasts.—Nucleated red blood-cells of normal diameter.

Oligochromemia.—A diminution in the normal amount of hemoglobin. This may occur either independently or coincidently with a diminution in the number of red blood-cells.

Oligocythemia.—A diminution in the number of red blood-cells.

Plethora.—An increase in the total quantity of blood above normal.

Poikilocytosis.—This term is applied to the very irregular shape of the erythrocytes observed in certain pathologic conditions.

Polychromatophilic Degeneration or Anemic Degeneration (Ehrlich).—This is an atypical staining reaction of the erythrocytes, the significance of which is not yet definitely determined.

Polycythemia.—An increase in the number of red blood-cells as compared with the fluid-content.

VARIETIES OF LEUKOCYTES.

1. **Normal Leukocytes.** — (a) Lymphocytes: These are derived from the lymph-glands, and appear as small, round cells about the size of a red blood-corpuscle, with a large, centrally located nucleus, and a small margin of protoplasm. The nucleus stains rather intensely with the nuclear stains (hematoxylin, methylene-blue, and Ehrlich's triple stain). The protoplasm is free from granules.

(b) Large Mononuclear Leukocytes.—These cells are two or three times as large as a red blood-cell, with a large, usually oval nucleus which is generally eccentrically placed. It stains poorly with nuclear stains. There is a relatively large amount of protoplasm, which is free from granules. They are derived from the bone-marrow, and may be regarded as the parent type of the polymorphonuclear.

(c) Polynuclear or Polymorphonuclear (Neutrophilic Leukocytes.—These are recognized by their multiple, irregular-shaped, or bent nucleus. The nuclei stain very intensely, and the protoplasm is densely packed with neutrophilic granules.

(d) Eosinophilic Cells.—These resemble the polymor-

phonuclear cells, except that the small neutrophilic granules are replaced by coarse acidophilic (eosinophilic) granules. These granules are highly refractive, so that they can be readily recognized without staining.

(c) Mast Cells.—These are cells of the polymorphonuclear type with marked basophilic granules, which are quite large, uneven, and irregularly distributed. They are not distinctly stained by the triple stain.

2. **Pathologic Leukocytes.** — (a) Mononuclear Neutrophilic Cells (Myelocytes): These are large cells with a large, faintly-staining nucleus, differing from the large mononuclear cells of normal blood by the presence of neutrophilic granules in the protoplasm.

(b) Mononuclear Eosinophilic Cells (Ehrlich's Eosinophilic Myelocytes): These cells are what their name implies, mononuclear eosinophiles. Very small cells of this type have been termed *eosinophilic microcytes.*

THE DIFFERENTIAL COUNT.

The distinguishing characteristics of the various white cells, as brought out by any one of the differential stains above outlined, allow the investigator to separate these into several groups, and thus to estimate their relative numbers which are usually expressed in percentage. The cells for a differential count can be enumerated without the aid of a mechanical stage, but this instrument of precision is a distinct aid in the performance of this procedure and should always be used when possible. Select a uniform and not too dense part of a stained smear, and adjust upon the mechanical stage so that the field presents cells in uniform arrangement without any overlapping. Now, by means of the thumb-screws of the mechanical stage, the field is carried back and forth before the eye of the observer, so that the same part of the field is not brought into view more than once (this matter is very simple when a mechanical stage is used). As the parade of cells passes before the eye, the white cells are observed, classified, and the number jotted down. This is continued until not less than two hundred, and preferably five hundred or a thousand, cells have been counted. With the total

number of cells counted known, and also the number of cells in each class recorded, it is a simple matter to calculate the percentage of each variety of cell in the specimen examined.

The Normal Differential Count.—The normal leukocytic count may vary between 5000 and 10,000 white cells per cubic millimeter. The average normal leukocyte count is usually placed at 7500 leukocytes per cubic millimeter.

The minimum and maximum number of cells for each type, estimated from the minimum normal (5000) leukocyte count, is given in the following table:—

VARIETY.	MINIMUM.	MAXIMUM.
Polymorphonuclears	3000	3500
Small lymphocytes	1100	1500
Large lymphocytes	250	350
Eosinophiles	50	100
Mast cells	5	25

The minimum and maximum number of cells of each cell-type estimated from the maximum normal leukocyte count (10,000).

VARIETY.	MINIMUM.	MAXIMUM.
Polymorphonuclears	6000	8000
Small leukocytes	2200	3000
Large leukocytes	500	900
Eosinophiles	100	200
Mast cells	10	50

For the average normal standard for each cell-type we may adopt the following standard:—

VARIETY.	NUMBER.	PER-CENT.
Polymorphonuclears	4875	65
Small lymphocytes	1950	26
Large lymphocytes	525	7
Eosinophiles	75	1
Mast cells	8	0.1

LEUKOCYTOSIS.

For convenience in the study of the various forms of leukocytosis, they may be divided into two classes: *(a)* Physiologic leukocytosis. *(b)* Pathologic leukocytosis.

(a) **Physiologic Leukocytosis.**—The average number of

leukocytes, per millimeter of blood, is normally 7500. For children the average is slightly more. For weak and poorly nourished persons slightly less. The numbers of leukocytes in the peripheral blood of any individual vary from time to time. They (1) may be increased after a hearty meal, especially if it contains much proteid material. Physiologic leukocytosis may also occur (2) during pregnancy, particularly during the latter months of the condition; (3) in the new-born, and (4) after cold baths.

In these so-called physiologic leukocytoses the increase does not usually exceed 30 per cent. of the normal, though in children it may be doubled.

A *hypo-leukocytosis* is said not infrequently to precede a hyper-leukocytosis.

(b) **Pathologic Leukocytosis.**—Many infections cause an increase in the number of white corpuscles in the peripheral circulation. Although the varieties of cells are the same as in health, the relative proportions are usually altered. In the most common form of pathologic leukocytosis, the percentage of lymphocytes is diminished, while the polymorphonuclears are frequently increased from normal (65 per cent.) to 90 or 95 per cent.

The *polymorphonuclear leukocytosis* occurs especially during inflammatory processes, and above all in those accompanied by purulent exudation.

In certain infectious diseases, notably typhoid fever and uncomplicated tuberculosis, there is usually no increase in the number of white blood-cells.

The origin of the extra leukocytes has not yet been definitely determined, as we do not know whether they are derived from the bone-marrow, lymph-glands, or from other tissues.

Another form of leukocytosis is characterized by a relative increase in the number of *eosinophilic* leukocytes. This condition of the blood is notably observed in bronchial asthma, trichinosis, and infections with other animal parasites. It is of interest to note that in these conditions there usually exist local collections of eosinophiles at the seat of disease. Thus, in the walls of the bronchi and in the exudate in bronchial asthma, and about the embryos in trichinosis.

The number of white cells in a pathologic leukocytosis not infrequently reaches 20,000 to 30,000 cells per cubic millimeter, and has been known to reach the enormous number of 168,000 (Grawitz).

Leukopenia.—A diminution in the number of leukocytes in the peripheral blood occurs in a variety of conditions. It has been observed in cachexias, in intoxications, many anemias, and in some infectious diseases, notably in typhoid fever and in malaria.

In leukopenia, as in leukocytosis, the relative proportions of the various varieties of white cells are usually changed. For example, in typhoid fever there is often a relative increase in the number of lymphocytes.

THE ANEMIAS.

Anemias are conveniently classified as **primary** when due to some unknown cause, and in which the blood-changes are, as a rule, of both a quantitative and a qualitative type.

And as **secondary**, when the cause is known and when the blood-changes are usually of a quantitative type only.

The Secondary Anemias.—Causes: Secondary anemia is observed after hemorrhage, during pregnancy, during chronic and constitutional diseases, and in poisoning, including that large and vague group of conditions comprising auto-intoxication. In chronic digestive disorders, malignant tumors, tuberculosis, syphilis, malaria, and in the different forms of helminthiasis.

The Blood-Changes. — The chief blood-changes in secondary anemia consist in a reduction of hemoglobin and a diminution in the number of red blood-cells. Mild forms show no other changes in the blood-picture. Severe forms show poikilocytosis, macrocytosis, and microcytosis. The extent to which these conditions are observed corresponds roughly to the severity of the anemia.

Further the red corpuscles often manifest a change in reaction to the ordinary staining reagents. They stain poorly, unevenly, and some parts of some cells refuse entirely to take the stain. This condition is termed polychromatophilia. This

change is no indication of the grade of the anemia, as it is observed in the mildest forms of secondary anemia.

The white cells in the simple anemias present nothing that is characteristic.

Summary.—The essential blood-changes in secondary, or simple, anemias consist in a diminution in the hemoglobin percentage, and in the number of red blood-cells. The red cells may show polychromatophilic degeneration and poikilocytosis.

The Primary Anemias.—General Considerations: Unlike the secondary anemias, the blood-changes in primary anemias, besides showing any or all of the modifications observed in the former, present very striking and characteristic alterations in the white blood-cells.

Progressive Pernicious Anemia.—In contrast to the process in simple anemia, in progressive pernicious anemia, blood-degeneration in certain portions of the blood-making organs, notably in the bone-marrow, takes place in a manner different from the physiologic. Consequently, in the blood-formative organs and also in the circulation, we note cells often in great numbers that are never seen in the normal blood. These pathologic elements are present in embryonal life; so in pernicious anemia we speak of the reversion of blood-formation to the embryonal type.

The Blood-Changes.—In a typical, well-defined case of pernicious anemia, the first glance at a well-prepared stained specimen of blood is sufficient to separate it immediately from the class of simple anemias. We find that a large number of erythrocytes have a diameter greater than normal (megalocytes: diameter 15 to 16 microns). These cells by their staining show a great richness in hemoglobin. Careful search always shows some megaloblasts, *i.e.,* nucleated (embryonal) red corpuscles. Normoblasts and microcytes are also in evidence. Other changes usually present are poikilocytosis, polychromatophilia, and granular degeneration.

The red corpuscles are always notably decreased, and may be less than 20 per cent. of the normal. The percentage of hemoglobin is also diminished, but practically always in a less degree than the red cells. On account of this condition the color-index is very frequently above 1.0.

CHLOROSIS.

Blood-Changes.—The blood when drawn flows freely from the puncture, and is markedly watery. Hemoglobin estimation shows a decided reduction in the percentage of hemoglobin without a corresponding deficiency in the number of red blood-cells. The color-index is therefore low, below 1.0, in contrast to the high color-index observed in pernicious anemia. This particular characteristic is not unique in chlorosis, as other forms of anemia may also show it.

Morphologically we find striking changes in the erythrocytes (many appear as macrocytes), which are pale and are without a distinctly pronounced central umbilication—the cells appear swollen. The particular cells have been designated "chlorotic" blood-corpuscles. Severe cases shows poikilocytosis, and nucleated red blood-cells.

Polychromatophilia and granular degeneration, which are the genuine phenomena of degeneration, are not observed.

The condition of the leukocytes is not uniform. The cells themselves do not show any characteristic changes in this disease.

The blood-plaques appear in markedly increased numbers, so that many groups of these cells appear in every field of the fresh preparation.[6] These are often very evident in the stained preparation, especially if a basic stain, such as methylene-blue, has been used.

LEUKEMIA.

Changes in the Number of Erythrocytes.—The blood from a marked case of leukemia is distinctly watery; in extreme cases it may be a whitish-red as it emerges from the puncture; this is owing to the great increase in white elements.

The microscopic examination of the fresh blood in established cases, shows even without counting the enormous increase in the white blood-cells. The count of the white corpuscles shows 100,000 to 500,000, or even more, to the cubic millimeter.

In some cases of leukemia no noteworthy change in the erythrocytes, either in number or appearance, occurs. As a rule

[6] E. Grawitz: Modern Clin. Med., p. 327.

they are decreased to about half the normal number. Beside the diminution in these cells the blood contains varying numbers of normoblasts, and more rarely megaloblasts.

The amount of hemoglobin is diminished, but the coloring of the individual corpuscles need not be diminished (no alteration in the color-index).

Changes in the Blood-Plaques.—An increase in the number of the blood-plaques has been noted in a number of cases.

Blood Morphology.—The diagnosis of leukemia can frequently be made without further consideration in cases which show an extraordinary increase in the number of white elements, but in doubtful cases the presence of the condition can only be determined by an exact examination of the morphology of the leukocytes.

Besides noting the great increase in the number of white cells, the polymorphonuclear elements will usually be found the most numerous.[7] In other cases the increase is chiefly among the lymphocytes. We may therefore differentiate two forms of leukemia.

1. Lymphatic Leukemia. 2. Leukocytic Leukemia.

Since the source of the lymphocytes is from the spleen and from the lymph-glands, and that of the leukocytes from the bone-marrow, the common designation of lymphatic leukemia (lymphemia) and myelogenous leukemia (myelemia) may be applied to these two basic forms of leukemia.

As, however, the bone-marrow normally produces typical lymphocytes, and as it has been demonstrated in rare conditions that an overflooding of the blood with lymphocytes may occur through proliferative changes in the bone-marrow without enlargement of the spleen or lymph-glands (myelogenous lymphemia). It seems preferable to apply the less prejudicial division of the leukocyte forms into "lymphatic leukemia" and "leukocytic leukemia" (W. von Leube).

Differentiation.—1. Lymphatic Leukemia: The blood-picture is conspicuous for its great preponderance of large and small lymphocytes, in comparison to the leukocytes. Nucleated red cells (normoblasts) and megaloblasts, although present in

[7] W. von Leube: Modern Clin. Med., p. 349.

this form of leukemia, are by no means as abundant as in the leukocytic form.

2. Leukocytic Leukemia.—This is by far the most common form of leukemia. It may easily be differentiated from the preceding by the entirely different blood-picture. The increase in the white elements is usually very marked. Here, however, the polymorphonuclear leukocytes are greatly increased in the microscopic blood-picture. Neutrophiles and eosinophiles are absolutely always increased (Ehrlich). There is also an increase in the mast cells, which may be twice as numerous as the eosinophiles. Their determination is of the greatest importance, since a marked increase in mast cells is observed only in this disease (von Leube).

The phenomena which especially characterize leukocytic leukemia, and which result from changes in the bone-marrow (myelogenous leukemia) are the occurrence of neutrophilic and eosinophilic myelocytes. These may be present in enormous numbers (up to 100,000 per cubic millimeter). The first sight of such a picture simulates the blood-picture of acute lymphemia with its large mononuclear cells.

The *myelocyte* is a large mononuclear cell with an irregular nucleus, surrounded by a considerable amount of protoplasm, in which are either neutrophilic or eosinophilic granulations. Besides these immature leukocytes (myelocytes), immature forms of erythrocytes, also originating in the bone-marrow, are found in the circulating blood-stream of patients suffering from leukocytic leukemia. There are also normoblasts and occasionally megaloblasts.

SPECTROSCOPIC EXAMINATION.

Solutions of hemoglobin and its derivative compounds, when examined with the spectroscope, give distinctive absorption bands.

The Spectroscope.—Light, when made to pass through a glass prism, is broken up into its component rays, giving the play of rainbow colors known as the spectrum. A spectroscope is an apparatus for producing and observing the spectrum. In brief, the apparatus consists of a base or stand, two horizontal

tubes, and a prism arranged to take the light coming from one and to pass it into the other.

Light falls upon the prism through one tube known as the "collinator tube." A slit at the end of this tube admits a narrow ray of light which, by means of a convex lens in the other end of the tube, is made to fall upon the prism with its rays parallel. In passing through the prism the ray of light is dispersed by unequal refraction, giving the spectrum. The spectrum thus produced is examined by the observer through the other tube, which is a telescope. When the telescope is properly adjusted the rays entering from the prism produce a clear picture of the spectrum. If the light used is lamplight, then the spectrum will be continuous, the colors gradually merging one into the other from red to violet. If sunlight is used the spectrum will be crossed by a number of narrow, dark lines known as "Fraunhofer lines." The position of these lines in the solar spectrum is fixed, and the more distinct ones are designated by the letters of the alphabet, A, B, C, D, E, etc.

If, while using artificial light or the solar spectrum, a solution of any substance which gives absorption bands, is placed in front of the slit so that the light is obliged to traverse it, then the spectrum, as observed in the telescope, will show one or more narrow or wide, black bands, which are characteristic of the substance examined and which constitute its absorption spectrum. The position of these bands may be located by describing their relation to the Fraunhofer lines.

While the cost of the spectroscope may prohibit its use by the general practitioner, it is to be found in many laboratories, and its use in certain cases, particularly in poisoning, is absolutely essential, since it enables the student to arrive at definite conclusions which cannot be reached in any other way.

For ordinary investigation the pocket spectroscope is all that is required. The detection of CO-Hb and of methemoglobin, the first occurring in carbonic-acid poisoning, the second in the various forms of intoxication, particularly with chlorate of potash. The smallest amount of hemoglobin or its derivatives may be demonstrated with certainty by this means in cases where ocular examination would leave uncertainty.

The determination of CO-Hb (carbon-monoxid hemoglobin)

may present difficulty on account of the similarity of the spectrum to that of O-Hb (oxyhemoglobin). The differentiation is only certain when we observe that the line of CO-Hb does not appear on the addition of reducing agents—for example, ammonium sulphate. The CO-Hb is a strong combination, while, on the other hand, the O-Hb is changed by the reagent to that of reduced hemoglobin.

BACTERIOLOGIC EXAMINATION.

Both diagnosis and prognosis may, in some cases, be helped by a bacteriologic examination of the blood. As there are quite a number of diseases in which the pathologic agents enter the blood, where diagnoses may thus be corroborated by a simple microscopic investigation of the blood, as in relapsing fever, malaria, and syphilis. In other cases, because of the small total number of organisms, or of the presence of others due to contamination, it is advisable to proceed by the more tedious but more certain bacteriologic methods.

THE CLINICAL VALUE OF BLOOD CULTURES.[8]

"Blood-cultures are most likely to be of value in the following diseases: Typhoid fever, pneumonia, other forms of pneumococcic infections, and the whole group of septic cases in which the sepsis is associated with wounds, pelvic diseases, abortion, puerperal infection, endocarditis, and local diseases of the throat and many other regions.

"In infections with typhoid bacillus, the pneumococcus, staphylococcus, and streptococcus, the results are usually positive.

"In a case of typhoid the examinations along bacteriologic lines are most likely to be successful between the latter part of the first and the middle of the third week. In sepsis due to the pyogenic cocci positive results are obtained in the majority of cases.

"Blood-cultures may clear up doubt in the presence of

[8] Edsall: quoting Longscope and Evans.

typhoid fever or pulmonary tuberculosis. They may also be of considerable use in obscure infections in childhood."

The Specimen.—In the preparation of blood-cultures it is advisable to puncture a vein in the arm under strict aseptic precautions to withdraw one or two cubic centimeters of blood, and to proceed immediately to prepare cultures or proceed with animal experiments.

Relapsing Fever.—The diagnosis of this disease is only positive when the spirilli are found in the fresh blood. As a rule this examination is easy, for the peculiar curved form of the spirillum precludes confusion with other organisms. Their numbers are usually large and their detection facilitated by their motility. In addition they stain readily with the various aniline colors (as carbol-fuchsin).

Malaria.—In this disease the microscopic examination is of extreme practical importance. The organisms may be readily detected in fresh blood at the bedside with the aid of a good microscope. The dried stained specimen, which is usually the more practical to procure in practice, will serve to detect them even when in but small numbers.

The specimen for examination is best taken in the afebrile period, shortly before the expected paroxysm. Previous to this the administration of quinine should be withheld for as long a period as possible.

THE SPIROCHÆTE PALLIDA.

Description of the Spirochæte.—The spirochæte is four-fourteenth microns in length, and at most only one-quarter of a micron in breadth; its form is that of a corkscrew with six to fourteen or even twenty-four turns. It is in active motion when examined in the fresh, and may show this activity for days in salt solution, when the tissues are kept at 20° to 27° C. (Beer).

Method of Obtaining a Smear (Roescher).—The surface of the sore is cleaned with alcohol, irrigated with salt solution, and dried. It is then scraped with a needle, care being taken to avoid drawing blood, but bringing out a good deal of serum. Smears are made from the serum, and in it the spirochæte will be found. Serum may be drawn from an enlarged gland by

means of a hypodermic needle and the smears made from this if desired.

The Giemsa Method (for preparation of stain see Appendix).—The films are fixed in methyl, or ethyl alcohol, and dried with blotting paper. The stain is then diluted with distilled water in the proportion of one drop to 1 cubic centimeter of water, and then poured on the specimen. For satisfactory results the stain should remain on the specimen for from one to twenty-four hours. The time for staining may be materially shortened by adding a few drops of a $\frac{1}{1000}$ solution of potassium carbonate to the diluting water. By this expedient the spirochæte may be demonstrated in fifteen minutes, though for the best results an hour's contact with the stain is advised.

Method of Oppenheim and Sachs.—The smears are dried in the air and then, without previous fixation, are flooded with an alcoholic solution of carbol-gentian-violet (for preparation of stain see Appendix). After flooding the preparation it is warmed until it steams for a few minutes only; it is then washed gently in running water, dried, and examined. The spirochæte are stained blue.

Method of F. C. Wood (fifteen minutes).—The smears are fixed in strong methyl alcohol and dried with blotting paper. The following stains are then applied in succession:—

First, a few drops of a $\frac{1}{1000}$ watery solution of yellow water soluble eosin are spread over the smear by means of a pipette. Second, four or five drops of a $\frac{3}{1000}$ aqueous solution of methylene azur II. The two colors are thoroughly mixed by spreading over the smear and by rocking the cover-glass. This mixture is allowed to act for ten minutes. The stain is then washed off by a strong stream of distilled water, the preparation dried between blotters, mounted, and examined by an oil-immersion. For mounting use xylol-dammar, as the ordinary Canada balsam bleaches these preparations. Usually any precipitate which forms can be removed with the stream of water. If especially clean and clear specimens are desired, the precipitate may be removed by momentary immersion in 95 per cent. alcohol, which should be immediately washed off with distilled water. By this method the spirochæte appear a light carmine color.

AGGLUTINATION REACTIONS.

The Phenomenon.—We owe a valuable diagnostic method to the knowledge derived from the law of immunity. We know that in the natural infections of man, as well as by animal experiments, peculiar changes occur in the blood-serum under the influence of specific bacteria or their soluble toxins. Among these altered sera we differentiate two classes: 1. The *antitoxic sera*. 2. The *bactericidal sera*. The first variety is represented by the serum containing the diphtheritic antitoxin; the second by the energetic bacteriolytic action of the blood of convalescents from cholera.

As another variety of the latter, the group of agglutinating sera may be included; these occur during the course of and after recovery from many infections. We are most familiar with this condition after typhoid fever.

The Widal Reaction.—Definition: Agglutination in the bacteriologic sense refers to the clumping and sedimentation of micro-organisms from homogeneous suspension by the action of a serum. This is the so-called Gruber-Widal reaction.

Normal serum may agglutinate many bacteria, as typhoid, colon, pyocyaneus, and dysentery, but not the streptococcus and a number of other organisms. This agglutination only occurs in low dilutions, though the typhoid bacillus has been known to agglutinate in as high a dilution as $\frac{1}{30}$, a point to be remembered in practical diagnosis.

Nearly all the bacteria, even the non-pathogenic, will produce agglutinating serum when injected, but not all have this power equally.

Technic.—Two methods: 1. The macroscopic, or naked eye, observation of the clumping and sedimentation of a homogeneous suspension of bacteria in test tubes. 2. The microscopic observation of the clumping of the organisms when the bacteria are mixed with serum and mounted in a hanging-drop preparation.

For the hanging-drop preparation it is necessary to have a slide with a saucer-shaped depression in the middle of one surface. A drop of the solution to be examined is placed in the center of a cover-glass, which is then placed drop-side down over

the depression, and the edges sealed by vaseline or paraffine. In this there is ample room for the motile organisms to swim about, and the loss of motility incident to the agglutination is readily observed.

The Culture.—When the organism to be tested grows rapidly, it is absolutely necessary to use a fresh, young culture, one which has been grown at room, not incubator, temperature, on agar or in bouillon for from eighteen to twenty-four hours. If the agar culture is used the bacteria may be washed from the surface by pouring into the tube 5 to 10 cubic centimeters of normal salt solution, and shaking vigorously. The resulting suspension is then ready for use. A homogeneous suspension is absolutely necessary to successful work. Accidental agglutination from insufficient dilution, must always be suspected and guarded against.

The Serum.—A blister may be used. A cantharides plaster from one-half to three-quarter inch square is placed on the abdominal skin and covered with a dressing. In about fourteen hours remove the serum with a sterile hypodermic syringe, or 0.5 to 1 cubic centimeter of blood can be drawn in the usual way from the lobe of the ear or the tip of the finger, and the serum removed after coagulation has occurred, or the serum may be immediately separated in a suitable tube in the centrifuge.

The custom of drying a few drops of blood on a piece of paper, as practiced in many municipalities where the specimens are sent by mail to a central laboratory, is very convenient, and in general practical, but it precludes the possibility of using definite dilutions, because the bacteriologist does not know the precise amount of serum or blood used. (See Illustration of Philadelphia Test Blank.)

Serum Dilutions. — Ordinary serum of dilution, 1 to 10 or less, will often agglutinate the typhoid bacillus and other organisms, while higher dilutions, 1 to 30 or 1 to 60, fail to act. The serum of the typhoid patient or convalescent, however, rarely fails to agglutinate in these dilutions.

It is usually held that dilution of 1 to 40 or 1 to 50 is sufficiently high to eliminate the possibility of false agglutinating reactions by normal serum, and at the same time sufficiently

low to permit of agglutination of nearly all the sera of typhoid cases.

Loop Dilution.—A convenient method of measuring small amounts of culture and serum is to measure by means of a fine wire loop, such as used in bacteriologic work. One loop full of serum placed on a slide and diluted with ten loops full of water, will give a dilution of 1 to 10. By this means minute quantities of serum can readily and quite accurately be reduced to the desired dilution.

If agglutination occurs in a microscopic preparation, we note, with the aid of the high power, that, in the course of from fifteen minutes to an hour as the micro-organisms swim about, a few which come in contact have a tendency to remain in this position. In the course of a few more minutes other organisms join this group and other groups form throughout the field; motility becomes gradually less until it ceases entirely in the typical reaction. The complete change occurs in from six to eight hours. No less than five bacteria must become permanently agglutinated to constitute a positive reaction. The test is most decisive when large masses of permanently agglutinated organisms are formed which can be seen under the lower powers.

IV.

OPSONIC METHOD.

The Theory.—Two elements come into consideration in the protection of the organism against invading micro-organisms. The leukocytes with their digestive ferments constitute one of these elements; the antibacterial substances in the blood fluids constitute the other.

(a) **Leukocytes.**—The leukocytes come into consideration in connection with the resistance of the bacterial infection, by virtue of the fact that they are capable of ingesting bacteria and of disintegrating these by intracellular digestion.

We may usually distinguish between "spontaneous" and "induced" phagocytosis.

"Spontaneous" phagocytosis is the process of ingestion and destruction which occurs in different media, as physiologic salt solution. It is a slow process and the bacteria ingested are in moderate numbers. It is also irregular, in that the number ingested of different species of bacteria varies.

Strikingly different from such spontaneous phagocytosis is the "induced" phagocytosis, which comes under observation when leukocytes are brought into contact with bacteria which have been, or actually are at the moment, subjected to serum action. The "induced" phagocytosis which occurs under these conditions is distinguished by the following facts:—

1. It is an exceedingly rapid process. 2. With hardly an exception every adult leukocyte is phagocytic. 3. When the supply of organisms is unrestricted the leukocytes will ordinarily fill themselves to absolute repletion. 4. The leukocytes will continue to digest bacteria in a concentration of salt solution which would entirely suppress "spontaneous phagocytosis."[1]

(b) **Antibacterial Elements in the Blood.**—The antibac-

[1] Sir A. E. Wright: Jour. Amer. Med. Asso., Aug. 10-17, 1907.

terial elements which are here in question are of "bacteriotrophic" properties, in that they turn toward and enter into combination with the elements of the bacterial body. Our knowledge of the modifications which are effected in the bacterial body under the influence of the bacteriotrophic substances in the blood-fluids, is far from complete.

This bacteriotrophic action may manifest itself in various ways: 1. The bacteria may be killed without being dissolved. 2. The bacteria may not only be killed, but also dissolved. We may group these activities as bacteriocidal and bacteriolytic effects. 3. The bacteria may be so altered as to agglutinate in the presence of salt (agglutination reaction). 4. The bacteria may be so affected as to be readily ingested by the phagocytes (opsonic effect).

These effects can be experimentally proven. We may assume then, that we have in the blood-fluids, in addition to bacteriocidal substances (bacteriocidins), also bacteriolysins, agglutinins, and opsonins.

Of these four varieties of bacteriotrophic substances, the opsonins appear to be the most important. We are justified in ascribing to them predominating importance, because it can be shown that the opsonic effect is exerted either by the normal or immune blood of every species of bacteria, while the bacteriocidal and bacteriolytic activities are exerted among pathologic bacteria, apparently only on the typhoid bacillus and the cholera vibrio. The opsonic effect is of further importance, because the effect can be accurately measured (Wright).

General Considerations.—The opsonic substances are essential to induced phagocytosis. They do not act upon the leukocyte (as a stimulant to the cell), but combine with the microorganisms, thus preparing them for the leukocytes. Hence the term Opsonin, from *opsono,* I cater for. The opsonins have been shown to be almost destroyed by heating to 60° C. for a short time. The opsonic power of the blood gradually disappears on standing, losing about 50 per cent. of its activity in five or six days.

When a condition of immunity is conferred upon a patient infected with staphylococci, the opsonic activity of the patient's blood is greatly augmented.

It is possible that there are present in the blood-plasma many varieties of opsonins, each concerned with combating one particular kind of microbic invasion.

The leukocytes of healthy or of diseased persons seem equally active when brought in contact with the same serum; hence the amount of opsonins present in the blood of an individual determines, according to the opsonic theory, his susceptibility to bacterial invasion.

The Technic.—To measure the resistance of a patient to a bacterial invasion, in other words, to find his opsonic index toward a certain micro-organism, special technic has been developed, the salient points of which will be briefly described.

Bacterial Emulsions. — Young cultures of rapid-growing bacteria, such as staphylococci, pneumococci, gonococci, and colon bacilli, are inclined upon agar-agar for a period of not longer than twenty-four hours in the incubator. They are then washed off with normal salt solution. After this has sedimented for a short time the upper whitish layer is removed with a pipette into a small centrifuge tube, and the larger clumps of bacteria removed by rotating for a few minutes. The supernatant layer which is still whitish and opalescent, is called the bacterial emulsion. An emulsion, if suitable for work, should contain the germs well separated and evenly distributed.

In preparing emulsions of tubercle bacilli, the culture is heated, then ground in a mortar with salt solution until finely divided, after which the mixture is centrifugated and the cloudy layer of emulsion removed.

Determination of the Strength of the Emulsions.—The method of Wright consisted in counting the actual number of germs in a measured amount of emulsion; the process is both difficult and tedious. McFarland and L'Engle have devised an apparatus for estimating the strength of bacterial emulsions, which is termed a "nephelometer." This consists essentially of a series of sealed glass tubes containing various strengths of barium sulphate solution. These serve as standards for comparison of the density of prepared bacterial emulsions. For the best results it has been found that the tube corresponding to the 5-per-cent. solution of $BaSO_4$ is the best. (For complete description of the nephelometer see end of this section.)

The permanency of the emulsions varies a great deal. Suspensions of gonococci should be used at once. Those of staphylococci within two days, while the emulsion of the tubercle bacilli may be kept indefinitely.

Washed White Corpuscles.—The finger or lobe of the ear is prepared and punctured as for the blood-count, and the blood as it exudes is allowed to fall directly into normal salt solution (0.85 per cent.) containing about 1 per cent. of sodium citrate. This decalcifies the blood and prevents it from clotting. The corpuscles are then completely precipitated by the centrifuge, and then alternately washed with salt solution and centrifugated until all trace of the sodium citrate is removed. After the final centrifugation the salt solution is decanted and the thin, upper layer of sediment, the "leukocyte cream," consisting for the most part of washed white corpuscles, is removed.

The Serum.—The blood is obtained in the usual way and collected in small glass tubes suitable to place in the centrifuge. These are rotated and the serum separated. In obtaining the normal serum care must be exercised to select a healthy subject, or, what is better, to obtain a mixed specimen from a number of subjects. This is "pool serum."

METHOD OF OBTAINING THE OPSONIC INDEX.

Special capillary pipettes, graduated in three equal divisions, are required for mixing the washed corpuscles, the patient's serum, and the bacterial emulsion. These are taken up into the tube in equal amounts and immediately blown out upon a microscope slide and thoroughly mixed. This mixture is again taken up into the tube and sealed in with the aid of a small Bunsen flame. The sealed tube is then transferred to an incubator of the ordinary type, or into an opsonic incubator as proposed by Freeman, and allowed to remain at a temperature of 37° C. for exactly fifteen minutes.

A second tube is prepared in the same way, only here normal or "pool" serum is substituted for that of the patient.

At the expiration of fifteen minutes smears from each tube are made upon cover-glasses and dried. These may be stained by any of the differential staining methods described in the section

on the blood. From the stained specimen a good field is selected (one containing a large number of leukocytes). The number of germs contained in the first fifty or one hundred cells is counted, and the total divided by the number of cells counted. This gives the the average number of bacteria per cell. Organisms which do not focus simultaneously with the cells should be excluded, as probably being extracellular. If the average number of bacteria per cell in the mixture containing normal serum was two, then the opsonic index for the normal serum is two. If the number of bacteria averages one for an equal number of cells in the mixture containing the patient's serum, then the opsonin index for that particular germ would be one-half.

Wright and his pupils, as a result of numerous observations, have classified diseases due to bacterial invasion as follows:—

1. Diseases in which the bacterial process is strictly localized or shut off from the lymph-channels, as furunculosis, lupus, and tuberculosis, and in fact almost any chronic infection. The opsonic index in these cases is constantly below normal, owing to the absence of immunizing stimuli emanating from the completely walled-off infective process.

2. Diseases in which the bacterial process is but loosely shut off, especially from the lymph-channels. In these, which are generally acute infections, immunizing agents from the bacteria, from time to time, get into the circulation; thus the opsonic index may be normal, above or below normal, at various times. A good example of this class is tuberculosis of the joints.

3. Diseases in which the bacterial infection is in the blood-stream. Here the opsonic index is usually above normal. To this class belong the infectious diseases: septicemia, endocarditis, etc.

4. Normal individuals not subject to any bacterial infection present a constant opsonic power to the various pathogenic bacteria.

For opsonic treatment of bacterial diseases *Wright* and *Douglass* lay down the following rules:—

1. Isolate in pure culture the causative micro-organisms.

2. Estimate the opsonic power of the patient's blood to that micro-organism.

3. If the opsonin index is at or below normal, as compared with normal or "pool" serum, prepare and standardize a vaccine from this micro-organism.

4. Inoculate the patient with this vaccine in appropriate doses at proper intervals, as shown by systematic examination of the opsonic content of the patient's blood.

Reflection will make clear that the success of the immunizing process must depend, in the first place, on the power of the immunizing response which the human organism may possess with respect to the particular bacterial infection or intoxication process which is in question, and, in the second place, on *(a)* the composition of the vaccine and *(b)* the dosage and method of administration.

STANDARDIZATION OF THE VACCINE.

The emulsions, with the exception of the tubercle bacillus, consist of dead cultures of the organisms (killed by heating). The amount of heat for sterilization will vary for different organisms: 60° C. or slightly less, for about thirty minutes, is usually sufficient. Cultures from the sterilized vaccines are then made to demonstrate that the culture is completely killed. The tubercle vaccine is the new tuberculin, "Koch." It is an opalescent fluid containing the active principle of the tubercle bacillus obtained according to the method of Koch, and may be procured in the open market.

THERAPEUTIC APPLICATION.

To successfully inoculate the prepared vaccine, it is necessary to have a pure culture of the organism, preferably grown from the patient's own strain of organisms. The patient's opsonic index to this particular germ is then taken. The patient is then given an initial injection of the standardized bacterial emulsion, and his opsonic index watched. In a short time after the inoculation the opsonic index will be found lower than it was originally. This is the so-called "negative phase." Sooner or later, from a few hours to a few days, the opsonic index rises above the starting point. This is the "positive phase." The

amount of opsonins in the blood now remains stationary for a while, then gradually diminishes. As soon as this beginning diminution is noted, a second injection of vaccine is given, which is again followed by a negative phase, though less pronounced than before. Following this a positive phase occurs which eventually reaches a higher level than the first. Thus the injections are repeated from time to time, according to the opsonic index of the patient, until the opsonic index in its positive phase reaches a normal or even higher level. In other words, if the injections are given properly (never in a negative phase), the patient's tissues are stimulated to an increased production of opsonins. The destruction of bacteria in the system is greatly increased, and the patient eventually recovers from the infection.

In the study of this process it will be noted that increased phagocytic response is associated with successful immunization, and that this phagocytic response is dependent upon an increase in the opsonic power of the blood-fluids and not on an increased capacity for spontaneous phagocytosis on the part of the white corpuscles (Wright).

When a dose of vaccine, which is only just sufficient to produce a result, is administered, the negative phase is elided and there is registered only a positive phase. When an excessive dose of vaccine is administered (one which produces severe constitutional symptoms) the negative phase is proportionately accentuated and prolonged.

The bacteriotrophic blood-power may be reduced for a period of weeks, and the advent of the positive phase in such a case awaited in vain. Immoderate negative phases due to overdosage, rarely come under observation except in the case of patients who are new to inoculation. In case a prolonged negative phase is produced by inadvertently administering an overdose, the desired raise can practically always be obtained by reinoculating as soon as all the constitutional symptoms have disappeared (Wright).

It is a proper principle of dosage in any series of inoculations, never to advance to a larger dose until it has been ascertained that the dose which is being employed is too small to evoke an adequate immunizing response (Wright).

A dose of vaccine may be adjudged too small as soon as it

has been ascertained that the inoculation is not followed by a negative phase, or when the negative phase is not very well marked and is of very short duration (Wright).

A whole array of observations point to the local production of bacteriotrophic and vaccinotrophic substances generally at the site of inoculation. It has also been noted that local immunity may be acquired and retained apart from the acquirement or retention of general immunity.

DIAGNOSIS OF OBSCURE LOCALIZED INFECTIONS.

Localized infections can be made to produce auto-inoculation by massage or by forced respiratory efforts in the case of pulmonary tuberculosis.

Auto-inoculation associated with measurement of the opsonic index is of value in the preliminary diagnosis of bacterial infections. When inoculation by this means can no longer be induced (which originally was possible), we may conclude that the focus is extinct, and *visa versâ*.

Conditions which Present the Simplest Problems. — (a) Penetration of a single species of micro-organisms into the body. Examples: *Tubercle* bacilli in *lymph-nodes*, *furunculosis*. In the treatment of these cases successful results are accomplished in a few days.

Tuberculin is a valuable aid to diagnosis in early tuberculosis, but when not properly used is dangerous, and in inexperienced hands may bring disaster.

The opsonic index may be of great aid in the early recognition of tuberculosis, especially where tuberculin cannot be given on account of temperature or prostration. The use of the opsonic index may give some indication for the control of the dose of tuberculin used for diagnosis as well as for its therapeutic effect.[2]

Grace Calvert thus sums up our present knowledge concerning the value of the opsonic index in pulmonary tuberculosis:—

1. In mild, early cases, if opsonic index is above normal, it is a favorable period of the disease.

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[2] Rotch and Floyd: Jour. Amer. Med. Asso., Aug. 24, 1907.

2. In acute cases it fluctuates greatly from day to day.

3. In chronic cases it is below normal.

(b) Ulcerative types of infection as in the breaking-down of the deeper tissues. This is not more difficult to treat than the preceding, except where secondary infection has occurred.

(c) Infections of the skin: *(a)* Those which are dry, scaly, and non-vascular; these are extremely intractable. *(b)* Those where the skin is vascular or where there is purulent infection, as in *acne* and *sycosis*. This form is very tractable to vaccine therapy.

H. R. Varney[3] reports 9 cases of acne in which no other method of treatment was employed. Six cases were cured. One discontinued treatment; two required other treatment in conjunction with vaccination.

A number of cases of furunculosis were cured with no recurrence in some months.

In two cases of sycosis of two and six years' standing, the first was not affected after prolonged treatment; the other was speedily cured.

(d) Infections of mucous membranes and glands and ducts connected therewith. These are readily influenced by bacterial vaccines when in the antrum, the nose, nasal sinuses, dental alveoli, salivary glands; also in infections of the intestines and gall-bladder, different infections of the uterus, urinary bladder, and urethra. Mixed infections add to the difficulty of treating these cases.

(e) Mixed infections. In a few instances the extinction of one species of bacterium by inoculation may lead to extinction of others which may be present. Such an event is extremely exceptional. More success follows efforts to immunize the patient against the different infections. This is particularly the case in lupus, cystitis, and in endometritis.

(f) Generalized infections. The vaccine method has favorably influenced some cases of *Malta fever*, and cures have been achieved in a few cases of *streptococcal septicemia* (Wright).

Therapeutic Immunizations in Mixed Infections.[4]—"Among the many problems that confront the physician who would apply

[3] H. R. Varney: Jour. Amer. Med. Asso., July 27, 1907.

[4] A. P. Olmacher: Jour. Amer. Med. Asso., Vol. li, No. 7, pp. 571-572.

in practice Wright's method of therapeutic bacterial immunization, is the one of mixed infection. In a general way it may be said that a mixed infection offers a less promising outlook than that of a single bacterial species, but still some brilliantly successful results have been obtained by inoculation in very complicated and long-standing infections.

"Given two or even three bacterial species, well known as pathogenic agents, and their simultaneous appearance in the secretion of a certain lesion, it is entirely proper to inoculate a mixed vaccine containing the proper doses of the offending bacteria; or, when the urgency is not too great, inoculation with the predominating and most likely pathogenic agent is first to be performed, and in the case of an unsatisfactory issue, a vaccine from the other bacterial species can be added to the subsequent injections; say, for instance, that staphylococcus aureus or a streptococcus is found associated with bacillus coli in the discharge from fistulas of abdominal origin, a mixed vaccine would be in order. . . .

"In dealing with mixed infections of a pyogenic nature, it is necessary to follow the events in the more chronic suppurations by bacteriologic analyses from time to time, and to modify the inoculations to correspond with the changes in the bacterial flora of the pus in case the therapeutic response is not satisfactory. . . .

"In dealing with tuberculous diseases in which a mixed infection is demonstrated by the culture-test, it is proper to combine the inoculations of tuberculin with vaccines of one or more of the complicating bacterial species. . . .

"Syphilis and a concurrent or a secondary infection with pyogenic bacteria, is another condition in which therapeutic bacterial immunization finds useful application."

THE NEPHELOMETER.[5]

With the Leishman or Wright method of estimating the phagocytic or the opsonic indices of the blood, it is essential to use bacterial suspensions containing uniform numbers of bacteria in order to secure uniform results. If the suspension used

[5] Jos. McFarland: Jour. Amer. Med. Asso., Oct. 5, 1907.

for one estimation contains twice as many bacteria as that used for another, the results will vary according to the density of the suspension. The method of Wright does not provide any means of preparing a suspension that shall have any given number of bacteria, so it seemed convenient to provide some method by which uniform suspensions could be prepared from time to time, as they might be needed.

The method suggested is that of using the density opacity of the suspension as an index of the number of bacteria contained, and devising some standard by which the opacity could be regulated.

It was this idea that materialized in the form of the simple instrument to which the name nephelometer has been applied.

It consists essentially of a series of standardizing tubes containing a suspension of a fine precipitate approximating bacterial suspensions in opacity and a holder for making their comparison easy. The standard suspensions used are precipitates of barium sulphate, made as follows: Two solutions, one a 13-per-cent. sol. of H_2SO_4 C. P., the other a 1 per cent. C. P. sol. $BaCl_2$, are prepared; then the one added to the other, so that 10 standards, containing 99 cubic centimeters of acid and 1 cubic centimeter of $BaCl_2$, 98 cubic centimeters of acid and 2 cubic centimeters of BaC.2, 97 cubic centimeters of acid and 3 cubic centimeters of $BaCl_2$, etc., are used.

According to the percentage or cubic centimeter of $BaCl_2$ solution used, these tubes are denominated 1, 2, 3, 4, 5, etc. About 3 cubic centimeters of each solution are sealed in a small test-tube, care being taken to use tubes of uniform diameter, and also to provide similar tubes for mixing bacteria to be compared with the standard.

Experience has shown that Standard 3 is most appropriate for staphylococci, and that the average phagocytosis for normal human blood when the corpuscles are mixed with this suspension and incubated for thirty minutes at 37° C., is 15 bacteria per polymorphonuclear. For the tubercle bacillus the most appropriate density is No. 5, and the average normal phagocytosis 3 bacteria per cell.

If the colorless barium sulphate solution is difficult to compare with the yellowish or brownish bacterial suspensions, this

difficulty may be overcome by placing a ground-glass screen before the tubes and comparing the suspensions by the use of artificial (candle) light.

The *modus operandi* of using the instrument is very simple: the standard-tube appropriate to the experiment is selected, well shaken, and placed in the left-hand pocket of the instrument, and the tube containing sterile salt solution stood in the other. The surface growth of the bacterium to be suspended is taken upon the platinum loop and stirred in, rubbing the bacterial mass on the side of the tube just above the surface of the liquid and gradually adding the fluid to it by tilting the tube so as to avoid suspending masses of culture. When the mixture appears homogeneous a comparison is made by looking through the instrument against the sky. If the density is insufficient more bacteria are added; if it be too dense, fluid can be added, drop by drop, from a medicine dropper, or, what is better, the tube can be given a few turns in the centrifuge, by which large clumps of bacteria are thrown down and it can be compared again. One should not be content with the comparison until the suspension be tried with others of the standard tubes than that which one intends to imitate. It is surprising at times to see how, what is thought to be an exact equivalent, turns out to be 1 per cent., more or less, dense.

When the density of a ready-made vaccine is to be determined, the procedure is varied by making the tube containing the vaccine the standard of comparison and changing the standardizing tubes until the density is fully determined.

Those who are constantly working in this field will do well to determine once for all the number of bacteria represented by each standard of density, and to make a little table for future use.

A modification that is sometimes to be preferred to the fixed standards is that of comparing the new suspension with previously-used suspensions, until exact uniformity is secured. The disadvantage of this method is that slight variations may pass undetected unless always compared with fixed standards, and that if the density of the suspensions used is not measured by some fixed standards, exact repetition by the experimentor himself, or by those who try to repeat his work, will be impossible.

V.

THE BLOOD-PRESSURE.

GENERAL CONSIDERATIONS.

Since 1733, when Stephen Hales first succeeded in measuring the blood-pressure in the arterial system with some accuracy, until a few years ago, the greater part of experimental work along these lines has been done upon the lower animals, particularly the dog. This has been by the direct method, which consists in exposing a vessel, inserting a cannula, and studying directly the circulatory changes by observing the fluctuations of some form of manometer connected with the blood-vessel through the cannula. Obviously this method of study, while applicable for examination of the changes in the vascular system from a physiologic or pharmacologic standpoint, does not represent the actual conditions existing in the human, and the data thus obtained can only be used to explain actual conditions in man by analogous reasoning. With the development of the indirect method of studying the circulatory phenomena, and the perfection of the various sphygmomanometers now obtainable, we are able to actually follow these changes as they occur in the human subject, and are able with great accuracy to note the effect of advancing disease, the effect of medication, and the intricate action of the nervous mechanism in both health and disease. Thus we have been able to refute or verify previous ideas and theories as applied to the arterial system, and have been able to supplement and augment the knowledge gained from ausculatory and palpatory methods; and, finally, to have ante-mortem diagnoses corroborated by the post-mortem findings in a far larger number of cases than heretofore.

Immediately following the practical development of the sphygmomanometer (Fig. 10) which is applied to the vessel *in situ,* and which affords a graphic demonstration of the pressure

and its variations in the vessel under observation, the study of the pulse and circulatory phenomena received a decided impetus which has steadily increased in strength, so that to-day a great mass of corroborative and explanatory data has accumulated, making the study of the pulse and of blood-pressure, and the many factors affecting and determining them, a very important chapter in clinical pathology, the practical importance of which makes it incumbent upon every physician to study and give careful consideration to this most important subject.

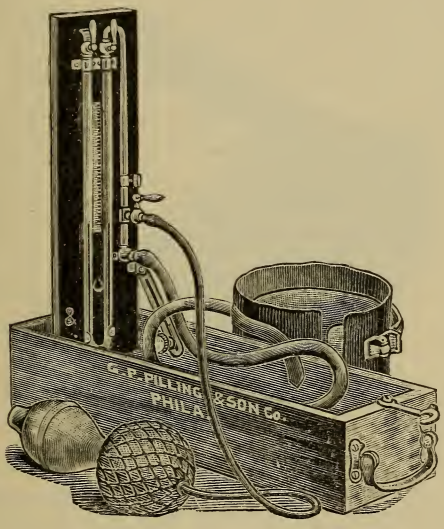

Fig. 10.—The Author's Sphygmomanometer. (Pilling & Co.)

Blood-Pressure in its Relation to Vessel-Contraction and Heart-Power.—Blood-pressure is determined by several factors: 1. The driving power of the left ventricle. 2. The condition of the channels through which the blood flows. 3. The condition of the flowing blood. In considering the question of blood-pressure, there must constantly be borne in mind the two principal factors affecting it. First, the physical laws which govern the flow of fluid through a system of tubes, and, second, the vital factor arising from the fact that the whole system is composed of living tissue and subject to the complicated mechanism which controls this.

Some of the physical factors are these: Starting from the left ventricle, the blood-pressure is highest in the aorta and falls gradually toward the capillaries. This is true of the flow of any fluid through a system of tubes. The fall in pressure as the periphery is approached, is the result of *(a)* friction between the flowing fluid and the walls of the tubes, and *(b)* the increased total area of the tubes on cross-section resulting from the repeated branching. Naturally a thick fluid will flow less readily than one which is thin.

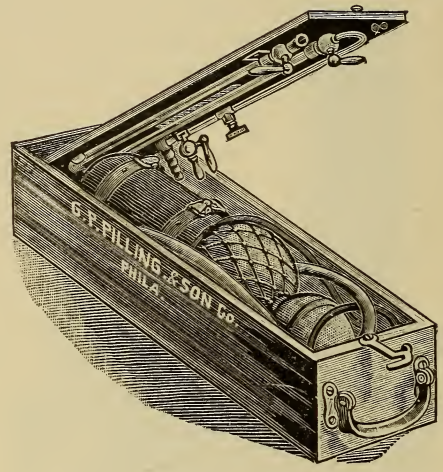

Fig. 11.—The Author's Sphygmomanometer, showing method of packing Arm-band and Bellows in case. (Pilling & Co.)

In studying the circulation the vital factor is by far the most important, frequently determining the physical changes. The vital factor is composed of, first, the inherent tendency of the vessel-wall to continue in a state of partial contraction—in other words, to have *tone,* and second, the central nervous mechanism controlling both the blood-vessel walls and the heart.

It is of greatest importance to separate these two factors when circulatory phenomena are considered, as a careful study and clear knowledge of the inter-relation of the factors of both classes will have much to do with a proper grasp of the situation which is necessary to an accurate prognosis and rational treatment.

Arterial blood-pressure is the pressure of the blood within the vessel under observation at the time of examination. It is chiefly affected by two factors. The state of the propelling mechanism and the condition of the circulating blood. The first of these is largely dependent upon the volume of blood entering the arterial system at each systole, and the resistance offered to the escape of blood from the arteries through the arterioles and capillaries.

In the clinical examination of the blood-pressure we must, first, constantly bear in mind the tactile sensation caused by the normal thickness of the vessel wall; second, the presence of increased tone or *hypertonus* producing a feeling of diminished diameter and increased thickness of the wall, and third, the occurrence of anatomic changes which may include atheroma, calcareous infiltration, or arteriosclerosis.

To those desirous of following this matter under the direction of a master mind, the little work, entitled "Arterial Hypertonus, Sclerosis and Blood-Pressure," by William Russell, is highly recommended.

THE SPHYGMOMANOMETER.

This instrument consists essentially of two parts, (1) a closed pneumatic system for applying indirect pressure to a vessel situated in an exposed part, usually the brachial. This pneumatic system is so arranged that air-pressure can readily be applied to the arm through the medium of an inflatable rubber cuff connected with the measuring device, and surrounded by a stiff outer or protective cuff of leather or reinforced canvas; (2) a mercury column by which the amount of pressure existing in the pneumatic system is accurately indicated upon the attached millimeter scale. The source of pressure is a small hand-bellows.

Method of Application.—To obtain the best clinical results with the sphygmomanometer it is important that all observations be made under as nearly invariable conditions as possible. This should be attained both in a series of observations on one case, as well as in differing cases, for the sake of more accurate comparison. Attention to this detail will, in a great measure, overcome variations in pressure resulting from changes in position of different parts, etc.

For general clinical study the arm-band (Fig. 10) should be adjusted to the left arm in the region of the deltoid insertion. By wrapping the rubber bag firmly about the arm and covering this closely with the retaining cuff, the danger of rupture of the inner cuff is avoided. In this connection a series of observations by the author serve to show that it is unnecessary to bare the arm before applying the cuff, as the interposition of a shirt or dress sleeve does not appreciably affect the reading. It is advisable, however, to remove the coat and to roll up one

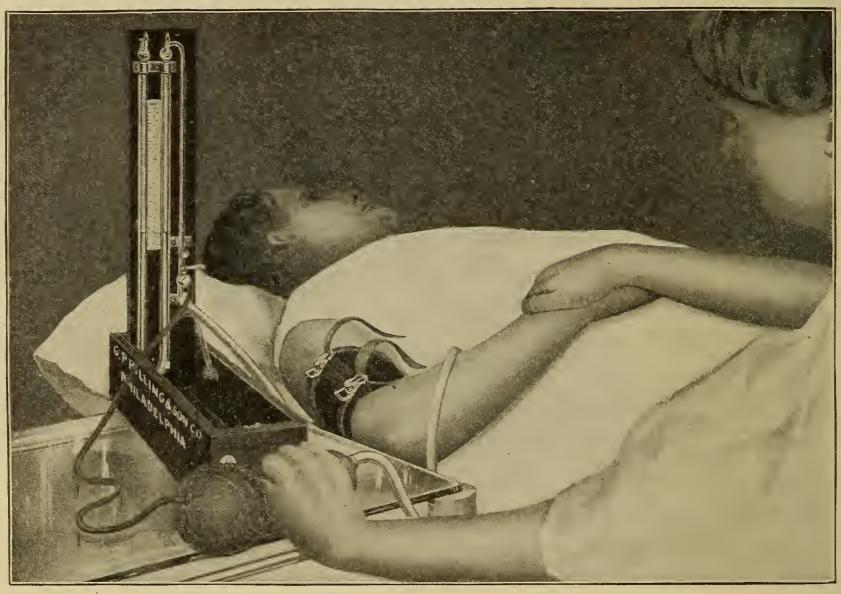

FIG. 12.—THE AUTHOR'S SPHYGMOMANOMETER, SHOWING CUFF APPLIED AND READY FOR BLOOD-PRESSURE DETERMINATION.

shirt sleeve when heavy winter underwear is worn. The tube emerging from the arm-band is connected with the sphygmomanometer by the branch tube, which is not supplied with the stop-cock. The hand-bellows is connected with the branch possessing the stop-cock; the needle-valve, situated immediately above this, is closed.

THE SYSTOLIC PRESSURE.—With these preliminaries completed the operator finds the most accessible part of the radial artery at the wrist. Here the general character of the impulse is studied, and the vessel carefully located, with the examiner's

hand in a position of ease. The pulse should be counted and the rate recorded; then, with the pulse still under observation, the air-pressure in the system is quickly raised until the radial pulse is completely obliterated. The application of more pressure than just sufficient to accomplish this is unnecessary, and only adds to the patient's discomfort. The stop-cock controlling the bellow's tube is then closed, and the needle-valve slightly opened, just sufficient to allow a gradual fall in the mercury column. As this fall occurs the examiner should be on the alert to note the return of the first impulse at the wrist. The height of the mercury column at this instant will exactly represent the measure of pressure required to obliterate the lumen of the artery under observation. In other words, the pressure in the pneumatic system approximates very closely the pressure exerted by the blood in the vessel under compression, and must, therefore, represent the pressure at its highest point during a heart cycle, *i.e.*, the *systolic pressure.* The height of the mercury column at this moment is jotted down, and a second observation made in the same manner to corroborate the finding.

The diastolic pressure may be obtained in one of two ways: First, after the systolic pressure has been determined, the fall of the mercury column is allowed to continue. As this occurs the mercury column will begin to show a slight oscillatory movement, which is synchronous with the pulse. At some point during the descent of the column of mercury, this oscillation will reach a maximum, after which it will grow less and eventually cease before the column reaches zero. The base of the column, when the movement is at its height, represents the diastolic pressure. Fluctuations of the mercury corresponding in rhythm to the respiratory movement, or irregular variations due to accidental muscular movement by the patient, must not be confused with the pulse wave.

In certain cases, particularly those presenting hypotension, it may be impossible to obtain the diastolic reading according to the above method. This is because the volume of the pulse wave or the force of the impulse is not sufficient to produce the necessary oscillation. In these cases the following method can usually be successfully employed:—

After obliterating the radial pulse as in the examination for the systolic pressure, allow the pressure to slowly and steadily fall, at the same time carefully keep the fingers upon the pulse. As the pulse first reaches the wrist it will be found to be weak and thready, which characteristic will continue for a variable time, until suddenly the force of the impact will become greater and will assume the character of the pulse of aortic regurgitation.

A reading of the height of the mercury column at the moment of this change in the pulse, will indicate the diastolic pressure.

The Systolic Pressure.—The systolic pressure, as indicated by the sphygmomanometer, represents the greatest amount of pressure to which the arterial wall is subjected during the contraction of the left ventricle.

The Diastolic Pressure.—The diastolic pressure represents the ebb to which the pressure within the vessel falls during the filling of the ventricles.

The Pulse Pressure.—This figure represents the difference between the systolic and the diastolic pressures, and represents the total variation in pressure to which the vessel wall is subjected during a complete cardiac cycle. Having determined the systolic and the diastolic pressures in any case, it is a simple matter to obtain the pulse pressure by subtracting the diastolic from the systolic figure.

The sphygmomanometer bearing the author's name (see Figs. 10, 11, and 12) has been recently devised, and embodies all the advantages possessed by the older models. Its individual points of merit are: First, the small size of the carrying case greatly reduces the total weight of the instrument, at the same time increasing its portability. Second, the simplicity of the mechanism and the absence of detachable parts reduces the time required of "setting up" to a minimum, at the same time making it impossible to lose or forget a part of the instrument during transportation. Third, the mercury employed in the manometer is never exposed, and therefore there is no opportunity for spilling, which might interfere with the accuracy of its readings. Fourth, the scale is adjustable and can instantly be regulated to the height of the mercury column.

FACTORS INFLUENCING THE PULSE PRESSURE.

It is theoretically possible that the pulse pressure should be increased in three ways, but it should also be remembered that clinically the condition of the pulse pressure bears a relation to all three, and that the occurrence of but one of the factors without some admixture of another, is a rarity.

1. An increase of the amount of blood delivered by the left ventricle into the aorta would tend, other things being equal, to increase the difference between systolic and diastolic pressures.

2. The same effect would be produced by any condition which would hasten the escape of blood from the vessels, independently of whether the blood escaped through the capillaries or was returned to the heart by regurgitation.

3. Undue rigidity of the vessel walls will exert a similar influence upon pulse pressure, as in factor two, because the harder and less elastic the arteries become, the more the heart is compelled to move the blood throughout the arterial system as a whole, while the diminished elasticity would, for mechanical reasons, cause the pressure during diastole to become very low.

METHOD OF RECORDING OBSERVATION.

For convenience in study, comparison, and for future reference, it is advisable to formulate and adhere to some method of recording the blood-pressure observations.

A chart which has been found satisfactory for this purpose, and which is designed to include all possible observations in this connection, will be found on page 281 in the Appendix.

POSTURAL VARIATIONS IN BLOOD PRESSURE.

Summary of observations made by O. Z. Stephens[1] on a series of twenty-two healthy medical students:—

SYSTOLIC PRESSURE.	STANDING.	SITTING.	SUPINE	HEAD DOWN.	RIGHT LATERAL.	LEFT LATERAL.
Right arm	132.6	133.3	152.5	166.2	155.0	110.0
Average	130.8	131.7	150.4	165.6	143.5	133.0
Left arm	130.0	130.0	148.3	165.0	114.0	156.0
Pulse rate	86	82	68.7	65.8	68.1	69.1

[1] O. Z. Stephens: Jour. Amer. Med. Asso., Oct. 1, 1804.

Summary.—1. The blood-pressure increases in the brachials from the standing to the head-down posture, in the following order: Standing, sitting, left lateral, right lateral, supine, and head down.

2. An increase in resistance is accompanied by an increase in heart strength.

3. The pulse rate decreases in the same order as the blood-pressure increases.

4. The decrease in pulse rate is a conservative act on the part of nature to protect the heart itself and the central nervous system.

5. The average systolic pressure in the sitting posture was found to be nearly one mmHg. greater than the standing position.

Physiologic Variations.—Besides the variations occurring in blood-pressure incident to alterations in posture, the following conditions have been studied in their relation to blood-pressure:—

Age.—During the first year of life the systolic pressure usually averages from 75 to 90 mmHg. In adults it ranges between 100 and 130. In the aged it lies between 130 and 145.

Excitement usually causes temporary marked rise in pressure.

Muscular exertion also increases systolic pressure. This increase becomes less marked as the individual becomes accustomed to performing the act or acts. The gradual reduction following systematic exercise is one of the benefits of training.

Sleep.— In the early hours of sleep there is quite a fall in blood-pressure, which gradually rises toward morning (Brush and Fairweather).

Pathologic Temporary Increase in Blood-Pressure.—*Drugs* may increase the blood-pressure either by acting upon the heart or by causing contraction of the peripheral circulation. *Acute asphyxia* or *acute anemia* of the brain will stimulate the vasomotor centers, causing a marked rise in blood-pressure.

Pain, even when slight or momentary, will cause a rise in blood-pressure even in normal individuals.

Pathologic Temporary Diminution in Blood-Pressure.—Widespread *dilatation* of the blood-vessels from any cause may

cause serious diminution in blood-pressure. *Lack* of *splanchnic tone* and wide dilatation of the abdominal vessels may have a fatal termination mainly through the great diminution in blood-pressure occasioned, the patient literally bleeding to death in his own abdominal vessels. A somewhat similar condition exists in *chloral* and *alcohol poisoning*, also in collapse.

Crile's exhaustive experiments seem to show that the condition known as surgical shock is caused by an exhaustion or paralysis of the vasomotor center, which renders it incapable of maintaining the normal vascular tone.

THE CLINICAL VALUE OF THE SPHYGMOMANOMETER.

The sphygmomanometer is especially applicable to the problems of medicine involving cardiac and renal disease, and should be constantly employed to furnish an index of the gravity of the condition, and as an aid in therapeutics by furnishing a graphic record of the effect of medication upon the cardio-vascular system. No pathologic condition of either the heart or the kidneys, or of the two in combination, can be thoroughly understood or rationally treated without a comparative study of the state of the circulation, blood-pressure, etc., and, without such a knowledge, the management of the case must of necessity be uncertain because of the element of guesswork, which can be eliminated in no other way but by employing this most valuable aid.

The great amount of painstaking work that has been done by many investigators along these lines, has resulted in the development of many clearly recognized and carefully classified clinical pictures, a knowledge of which will often lead in the right direction by shedding much light upon the inter-relation of apparently dissociated facts.

In Cardiac Disease.—The sphygmomanometer finding alone may be sufficient to determine the diagnosis. In *aortic regurgitation* the large volume of blood projected into the aorta by each systole must of necessity produce a high systolic reading, while subsequently, owing to the disposal of blood in two directions, through the capillaries and back into the left ventricle, the diastolic fall will be great. The combined result of these two

extremes will produce an enormous pulse pressure, this difference becoming even greater in the presence of markedly sclerotic and hardened arteries.

Therapeutically, we are enabled more accurately to follow the action of the drugs employed. Thus we obtain the fullest effect from the measures available, without overtreating, and are at the same time guarded against the administration of improper or useless remedies.

In the Diagnosis of Heart-Muscle Degeneration. — The sphygmomanometer is frequently the earliest means of detecting diminished heart-power from beginning degeneration of the muscle, this evidence being readily obtainable long before the development of the usual physical signs. This condition may be detected in the following manner: After applying the instrument, while palpating the pulse at the time when the pressure is gradually being raised, it will occasionally be noted that just before obliteration of the pulse some systoles fail to reach the wrist, showing undoubted irregularity in the power of the heart's action, the weaker impulses being prevented by the obstruction from reaching the wrist. This phenomenon can be very readily demonstrated in a pulse which to ordinary palpatory methods is apparently regular in force and rhythm.

In Renal Disease.—Here we may be enabled to confirm the diagnosis of incipient or latent Bright's disease by a study of the blood-pressure. In the presence of a slight recurrent albuminuria with beginning arteriosclerotic change and a systolic pressure which remains persistently above the normal in spite of appropriate treatment directed toward the hypertension, the probable diagnosis would be that of beginning renal change. Thus by an early warning we may be enabled to check the advance of serious symptoms or at least to considerably prolong the patient's period of activity and usefulness.

In cases of both acute and chronic lesions of the kidneys, it will be almost uniformly noted that the blood-pressure, as indicated by the sphygmomanometer, is permanently increased. In chronic cases the systolic not infrequently reaches 200 or 225 mmHg., while occasionally it may be above 250.

Friedlander[2] states that if this high pressure lasts for more than four weeks, hypertrophy of the heart is sure to ensue.

[2] Friedlander: Arch. f. Physiol., 1901.

In primary acute Bright's disease and in nephritis following scarlet fever, there is probably always an increase in arterial pressure. Sudden and marked rise in pressure has been noted within forty-eight hours of the onset of an acute nephritis.[3]

In advanced nephritis the study and detection of high tension and hard pulse will indicate the urgent necessity for energetic treatment for the reduction of blood-pressure; thus the brain may be saved from the serious effects of continued high pressure, and the patient protected from the occurrence of coma or apoplectic attacks.

In angina pectoris the attacks may be lessened in number and severity by timely detection of the increased tension resulting from arterial spasm, the effect of diet, and medication can be followed; and as have been reported, under favorable conditions, a few patients will be, to all intents and purposes, cured.

Diabetes: Its Relation to Cardiac Complications.[4]—In a series of 25 cases, all ages, using both Stanton's and the Riva-Rocci apparatus; 12 and 18 centimeter arm-bands; 150 observations. Diastolic pressure hard to obtain.

Summary of Systolic Pressure Records.

Number of cases	25	
Average age	45	years.
Male	13	
Female	12	
Average weight	156	pounds.
Average systolic blood-pressure	127	mmHg.
Average of cases showing 3 per cent. sugar	121	mmHg.
Showing less than 3 per cent. sugar, pressure average	135	mmHg.
Number of cases developing acid intoxication	10	
Average systolic pressure	107	mmHg.
Number of cases not showing acid intoxication	15	
Average systolic pressure	140	mmHg.
Number of cases showing indication of arterio-sclerosis and kidney involvement	5	
Average systolic pressure	164	mmHg.

Exophthalmic Goitre.[5]—The systolic pressure varies, sometimes low, usually higher than normal, occasionally very high. The pulse pressure may be very great, showing probably large systolic output from the left ventricle.

[3] Buttermann: Arch. f. Klin. Med., Vol. lxxiv, p. 1.
[4] Arthur B. Elliott: Jour. Amer. Med. Asso., July 6, 1907.
[5] Reported by L. F. Baker: Jour. Amer. Med. Asso., Oct. 12, 1907.

Lead Colic and Beginning Peritonitis.—These two conditions are usually associated with high arterial pressure.

Scarlet Fever. — The blood-pressure curve follows very closely the pulse and temperature curve. After the seventh or eighth day the blood-pressure is usually below normal. Complications have a marked effect upon blood-pressure. Signs of increased arterial tension are present in most, if not all, cases which show albuminuria; the high pressure is usually synchronous with the onset of the albuminuria. This is always accompanied by a marked slowing of the heart's action. With the subsidence of the nephritis the pulse-rate increases in the ratio that the arterial pressure diminishes.[6]

Diphtheria.—A reduction in blood-pressure will be found in every case, depending to a large extent, if not entirely, upon the degree of toxemia. The administration of strychnia and alcohol annul the fall of pressure, but only if given regularly.

The administration of antitoxin, while causing a slight rise in temperature, has no effect upon the blood-pressure (J. Davidson).

Typhoid Fever.—Here the blood-pressure is always considerably lowered, the minimum depressing steadily the longer the duration of the case. The pressure rises gradually to normal with the establishment of convalescence or through the interoccurrence of a complication, as pneumonia or peritonitis (J. Davidson).

THE EFFECT OF BLOOD-PRESSURE ON THE FLOW OF URINE.

The amount of urine passed varies directly with the pulse pressure.[7] High arterial pressure, by sending more blood to the kidneys, increases the flow of urine.

Lowered general arterial pressure will cause a diminution in the quantity of urine; this diminution is augmented if for any reason there be also an increase in venous pressure.

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[6] J. Davidson: Lancet, Oct. 19, 1907.

[7] Erlanger and Hooker: Amer. Jour. Physiol., Vol. x, p. 7.

VI.

COAGULATION TIME.

GENERAL CONSIDERATIONS.

The results obtained by improved clinical methods have only recently been reduced to a sufficiently accurate basis to admit of much confidence being placed in them, or to permit of their use in clinical medicine.

The time required for coagulation of the blood depends upon many conditions. This fact makes attention to detail and uniformity of technic of the utmost importance.

The chief factors influencing the coagulation-time are: 1. The time during which the shed blood remains in contact with the tissues, coagulation being slower from a deep than from a superficial incision, the difference in time may amount to as much as three minutes (Emerson). 2. The amount of pressure applied to the tissues to start the flow. 3. Upon the amount of blood shed. 4. Upon the nature of the vessel receiving the blood and its temperature. Factors of less importance are: time of day, atmospheric humidity, relation of time of shedding to digestion, ingestion of drugs, etc.

THE SPECIMEN.

This should be taken midway between meals, and not after the ingestion of drugs, which may influence coagulation-time. The tip of the finger or the lobe of the ear should be thoroughly cleansed and dried, and a sufficiently large puncture made with a flat-bladed instrument (Daland lancet) to insure free and sufficient flow.

METHODS OF DETERMINATION.

Milan's Method.—Place a drop of blood upon the center of a large glass slide or watch crystal. After a minute or two gently

tilt the glass from side to side. This is continued at short intervals until the drop of blood, which at first assumed a pear shape when the glass was tilted, is seen to assume the form of a blunt and symmetrical cone. This change in the character of the drop indicates the completion of coagulation. The average time of coagulation of normal blood by this method is about five minutes.

This method is at best imperfect and uncertain, since none of the modifying factors are controlled.

Wright's Method.—This is a far more accurate method, since in a large measure it controls and renders uniform the majority of the variable factors.

Wright's coagulometer consists of the following parts (Fig. 13) :—

Copper reservoir with numbered grid and rubber mat.
Pinchcock and tube.
Thermometer marked 18.5° and 37° C.
Twelve pipettes numbered 1 to 12.
Wire for cleaning tubes.
Lancets in bottle.
Aspirating tube.
Bottles for ether and alcohol.

The Principle of the Method.—A series of tubes of standard caliber filled with a definite amount of blood. The coagulation is observed at a standard temperature, and the coagulation-time is determined by blowing the contents of each tube upon a piece of clean white blotter or filter paper, at increasing intervals from the time of filling.

The tubes are 10 centimeters in length and have an internal caliber of 0.25 millimeters; they are marked at a point 5 centimeters from the lower end of the tube (*Fig. 1*). The coagulation-time is usually determined at 18.5° C. This temperature has been chosen as a standard because it approximates the temperature of the hospital ward or bed-room in temperate climates, and because this temperature delays the coagulation sufficiently long to allow the coagulation-time being, even in cases of rapidly coagulating blood, determined with considerable accuracy.

In case of slowly coagulating blood it is convenient to carry out the determination at 37° C. (blood-heat).

The tubes are to brought to a standard temperature before they are filled with blood. For this purpose close-fitting rubber caps are put on both ends of the coagulation-tubes, which are then placed butt end downward in the reservoir containing water of the standard (18.5° C.) temperature (see *Fig. 2 A*).

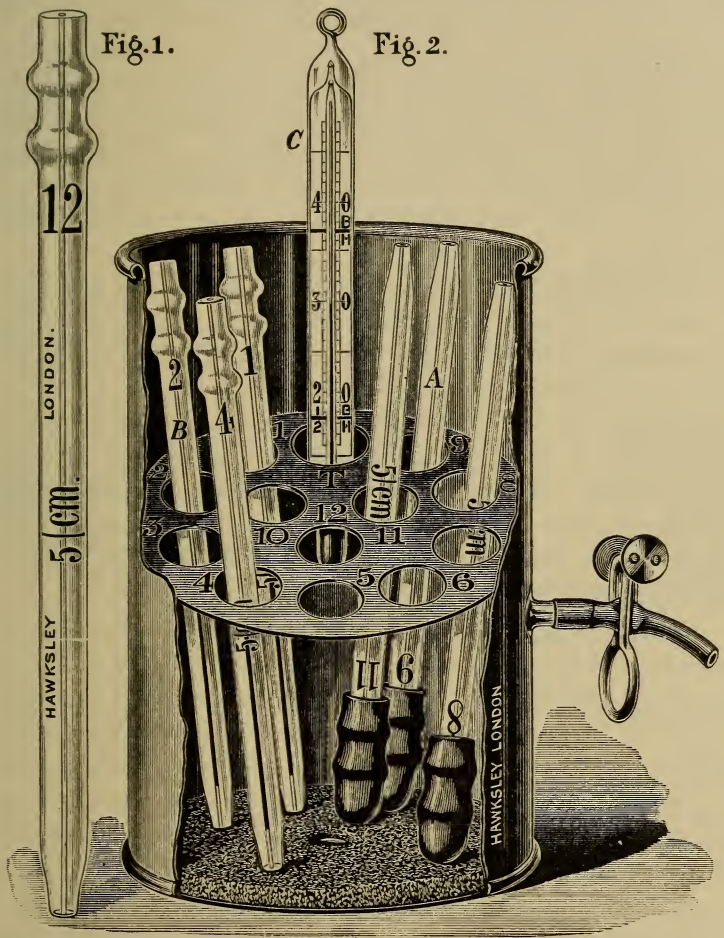


Fig. 13.—Wright's Coagulometer. 1. Capillary Tube. 2. Copper Reservoir. A. Capillary Tube cooling. B. Tube filled with blood. C. Thermometer. (Hawksley.)

After sufficient time has elapsed to allow the tubes to acquire the desired temperature, they are removed from the water and the rubber caps removed. Great care should be exercised to prevent moisture from entering the bore of the tubes.

Method of Filling the Tubes.—The blood which flows from the puncture is drawn into the tube with the least possible delay. A column of blood approximately 5 centimeters in length, is drawn into each tube, and then this column drawn a few centimeters from the tip of the tube. No important fallacy will arise if the column of blood be slightly longer or shorter than the 5 centimeters.

Before beginning the observation it is well to have the tubes arranged in the order in which they are numbered. In ordinary cases from six to eight tubes are employed. For cases where coagulability is greatly reduced, a larger number of tubes will be required, unless the coagulation-time has been approximately determined by a preliminary test.

A watch provided with a second-hand, and paper and pencil for noting the time of filling and blowing out, should be at hand. If an assistant is available, the task of keeping record of the time may be conveniently committed to him.

It is of decided advantage, before commencing, to set the second-hand so that it coincides with the minute markings around the dial. As each drop of blood is drawn, the time (minutes and seconds) is recorded.

It will be found convenient to allow an interval of ten or fifteen seconds between the filling of successive tubes.

As each tube is filled it is returned to the tank. It is not necessary to replace the rubber caps, as the air-space at the top of the tube is sufficient to prevent the water coming in contact with the blood in the tube. The viscosity of the blood will be sufficient to prevent the blood being forced further up the tube.

During the time that the tubes remain in the reservoir the temperature of the water must be maintained by the addition of hot or cold water from time to time as necessary.

After the lapse of exactly two minutes from the filling of tube No. 1, it is removed from the water and its contents blown upon a piece of white blotting or filter paper. If the contents cannot be blown out coagulation is noted down as complete. If the contents can be blown out, but shreds of fibrin are seen adhering to the interior of the tube, coagulation is recorded as incomplete. If the contents can be blown out cleanly and immediately sink into the paper, coagulation has not yet begun.

If in the first tube (two minutes) coagulation has been found to be complete, the second tube is removed at the expiration of one and three-quarter minutes, and the contents treated in the same manner. If this also is found clotted, the next in the series is tested at a still shorter interval, and so on until a tube is found in which coagulation is incomplete.

If the blood in the first tube, after the expiration of two minutes shows coagulation to be incomplete, a slightly longer interval is allowed before the second tube is blown out, and so on until one is found which shows coagulation to be complete.

The same process is carried out if, in the first tube coagulation has not yet begun, except that a still longer interval is allowed to pass before the second tube is blown out.

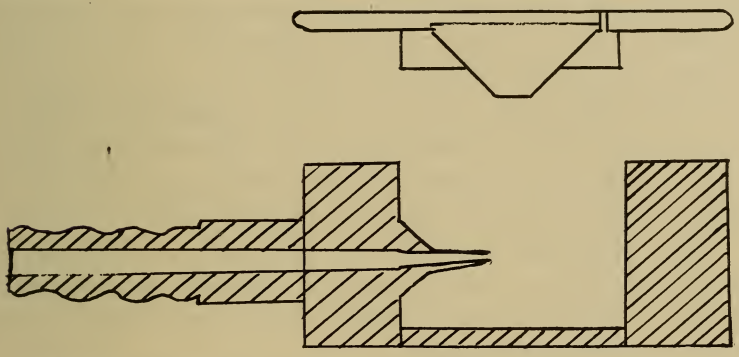


FIG. 14.—BOGG'S COAGULOMETER, DIAGRAMMATIC REPRESENTATION.

PERMISSIBLE MARGIN OF ERROR.—If the directions which have been outlined above are adhered to, the margin of error in the coagulation-time should not be more than five seconds, and should never exceed fifteen seconds.

The normal coagulation-time by this method lies between three and five minutes.

Method of Russell and Brodie.—This method requires a microscope. The coagulometer consists of a small, moist chamber with a glass bottom (Fig. 14), which can be placed upon the stage of the microscope. This is fitted with a truncated cone of glass which projects downward into the moist chamber. The end surface of this cone is of definite size (about 5 millimeters

diameter), and on it is placed the drop of blood, care being taken to see that the drop of blood only just covers the surface, hence is always of the same size. The cone is then quickly fitted into the moist chamber to prevent alterations due to drying, temperature, etc. Through the side of the moist chamber projects a fine tube, through which, by means of a hand-bulb, a gentle stream of air can be projected against the blood. The preparation of the slide being completed, the drop of blood is observed with the low power of the microscope.

The blowing should be done at as long intervals as possible, and also very gently. The corpuscles will first be seen to move freely as individuals; later, as coagulation begins, the corpuscles will no longer move freely in the drop, but the drop will begin to change shape en masse. Finally, there will occur only elastic motion, and the part of the drop displaced by the current of air will spring back to its original position as soon as the current of air ceases. This is to be taken as the terminal point, as now only can the clot be demonstrated, by quickly removing the slide and touching the drop to a piece of dry filter paper.

This apparatus is probably the best method yet devised for the determination of coagulation-time. Recently this instrument has been improved upon by Boggs, who has substituted a metal tube and an improved cone.

With the improved instrument Emerson (clinical diagnosis) reports normal variations in the coagulation-time between three and eight minutes, with an average of five minutes, eight seconds.

CLINICAL OBSERVATIONS.

The following results were obtained by Drs. Robertson, Illman, and Duncan with the Wright apparatus[1]:—

The normal clotting time was found to be from two minutes, thirty seconds, to two minutes, forty-five seconds, using half blood-heat (18.5°C.).

Typhoid Fever.—In the acute febrile stage the average clotting time obtained from observations on 61 cases was three and one-third minutes. A lengthened clotting time was found

[1] Report of Section on Pharmacology and Therapeutics, Amer. Med. Asso., page 51, *et seq.*, 1907.

to indicate impending hemorrhage. If, after hemorrhage, the usual shortening of the clotting time does not occur, then another hemorrhage is imminent. This shortening of coagulation-time is also noted after hemorrhage accompanying abortion and carcinoma of the uterus.

In typhoid fever the clotting time returns gradually to normal, as the temperature declines with the establishment of convalescence.

In cases showing abnormal clotting time, the administration of the many drugs supposed to favor this condition, both separately and combined, were found to have no appreciable effect upon the condition.

The following averages were obtained from observations on a large number of patients:—

Specific meningitis	3.10
Coal gas poisoning	4.05
Gastric ulcer	4.10
Salpingitis	5.15
Alcoholic gastritis	3.10
Apoplexy	3.05
Carcinoma	4.35
Jaundice	6.30 to 8.30
Rheumatism	3.00 to 4.00
Pneumonia	2.47
Nephritis	2.25
Diabetes	2.55
Exophthalmic goitre	2.45

VII.

BLOOD PARASITES.

THE PLASMODIUM MALARIA.

THREE forms or varieties of these organisms are recognized: the tertian, quartan, and estivo-autumnal. The cycle of growth of each of these differs. As the characteristic organism of the group, the plasmodium of tertian fever alone will be described, together with a brief differential table.

The Tertian Variety.—In the early stage this is a small, hyaline body with somewhat indistinct outlines occupying only a small portion of the interior of the red blood-cell, and usually situated eccentrically. It is living, and exhibits rapid and distinct ameboid movements. The hyaline body rapidly increases in size. Small groups of actively moving, pigment granules appear about this body, and the surrounding red cell becomes pale and swollen. When the parasite has attained its full growth, the outline of the red cell is hardly distinguishable. The activity of the ameboid movements gradually diminishes, and the pigment granules arrange themselves around the circumference of the cell. The final stage of development then begins. Signs of segmentation appear around the periphery of the organism, and the pigment granules pass toward and collect in the center. Finally, when segmentation is complete a "rosette" is formed by the symmetrical arrangement of the segments, sixteen to twenty in number radiating from the central mass of pigment. The tertian parasite passes through this cycle in forty-eight hours.

Quartan Variety.—The preceding description answers, except that the organism is smaller, the pigment less active and coarser. The segments number five to twelve, and the cycle of development is complete in seventy-two hours.

Estivo-Autumnal Parasite.—This organism also begins as

a hyaline body and develops in a similar manner. The parasites are smaller, the pigment granules scant and motionless. Segmentation occurs in the internal organs only, so that these forms are never found in blood obtained from the peripheral circulation. The organism requires twenty-four to forty-eight hours for its cycle. After the disease has lasted a week or more, pigmented crescents and spherical or oval bodies are found, which appear to be pathognomonic of this variety of parasite.

Apart from the usual life cycle, each form is capable of giving rise to extracellular, vacuolated, and flagellate bodies.

TERTIAN.	QUARTAN.	ESTIVO-AUTUMNAL
Develops in 48 hours.	Develops in 72 hours.	Develops in 24 to 48 hours.
Pale and indistinct.	Sharp outline, refracts.	Has irregular appearance.
Actively ameboid.	Slightly ameboid.	Actively ameboid.
Pigment fine.	Pigment coarse.	Pigment granules very few.
Pigment actively motile.	Pigment slow in movement.	Pigment still.
Pigment light.	Pigment dark.	Pigment light.
Full-size of corpuscle.	Smaller than corpuscle.	Smaller than corpuscle.
Degeneration forms twice the size of corpuscle.	Degeneration forms vary, much smaller than Tertian.	Absent.
Segments 16 to 20.	Segments 5 to 12.	
Irregular segments are frequent.	Beautiful rosettes.	Segmentation forms not seen in peripheral blood.
Corpuscles large, colorless and swollen.	Corpuscles shrunken and brassy.	Corpuscles shriveled, brassy, not decolorized.
		Forms crescents.

It is evident that while there is considerable similarity among the three varieties of parasites, there are, nevertheless, certain peculiarities and characteristics which enable the expert to quite positively differentiate between them.

THE DETECTION OF THE PLASMODIUM.

In searching for the organism it is always desirable, when possible, to examine the fresh specimen at the bedside of the patient. If this is impracticable, warm the slide and seal the cover with a little vaseline, and the organisms will retain their motility for at least a couple of hours.

If considerable time must elapse before the examination, films should be made, dried and stained, by one of the polychrome (Romanowski) stains.

Cautions.—Do not have the blood-films too thick; the individual cells should not overlap; there should be no rouleau formation. Do not give up the search until all the films have been examined thoroughly. A number of fields may show no organisms, then some may appear. The specimen is best obtained either eight or ten hours before or after a chill, as at this time the organisms are most likely to be in the peripheral circulation. Medication should be withheld for as long a time before the specimen is taken as possible.

What to Examine For.—Fresh specimen: Look for red cells containing actively moving black specks (pigment granules and living protoplasm). Unusually pale cells containing clear areas which are irregular and constantly changing shape. Extra large red blood-cells.

After a little experience the pigmented organisms are readily distinguished. Violent commotion among a group of red cells will direct attention to the flagellate. The hyaline bodies are usually the most difficult to identify without experience. The apparent, but not real (artefact), may be found in large numbers; these are usually distorted and deformed cells that have become altered in the preparation of the specimen. Finally, pigment-bearing leucocytes may be found in excess in malaria. These are, as a rule, polymorphonuclears, which have become phagocytic and have taken up the iron pigment, set free by the malarial organisms.

THE CLINICAL RELATIONS OF THE PLASMODIUM.

Segmentation of the organisms immediately precedes the chill and fever, consequently the segmentation forms will be present in the peripheral circulation at the beginning and at the height of the paroxysm, while the hyaline forms are discoverable during and after the seizure.

As the *tertian parasite* requires forty-eight hours to develop, segmentation and parasites will occur every other day, provided that only one group or brood of parasites be present. More commonly two groups of different ages exist (double tertian), seg-

mentating on alternate days and producing the quotidian (daily) type of fever.

The *quartan parasite,* requiring seventy-two days to mature, gives rise to a paroxysm with a two-day interval of ease. If there are two broods there will occur two days of paroxysm, followed by one day of rest (double quartan), while three groups will cause a daily or quotidian attack like the double tertian. In these multiple infections the time of fever need not occur at the same hour on successive days.

The *estivo-autumnal* type has variable periods of development. Segmentation and fever may be very irregular, and in some cases continuous.

SPIROCHÆTE OF RELAPSING FEVER.

The specific organism of relapsing fever may be found in the fresh specimen of blood. Prepare this in the same manner as for the malarial parasite. This organism appears as narrow spiral filaments from 36 to 40 microns in length. They are actively motile, and attract attention by the active movement imparted to the red blood-cells in their vicinity. They are in the peripheral blood only during the fever, when they appear in large numbers. Many may be seen in a single field of the microscope.

FILARIA SANGUINIS HOMINIS.

There are several forms of this parasite; for special distinguishing characteristics, larger works must be consulted. (See also page 113.) The most important of the group is the **filaria nocturna,** which is supposed to be responsible for certain forms of chyluria, elephantiasis, and lymph-scrotum. The adult or parent organism is slender and thread-like, varying from three to six inches in length. It inhabits the lymphatics and tissues. The embryos average in length about one-seventy-fifth of an inch, and are about as wide as a red blood-cell. The search for the embryos should be made both in the day and the night. Study under low power a fresh drop of blood. These embryos are decidedly active, and create a commotion among the neighboring cells.

TRYPANOSOMIASIS.

This is the organism of the so-called sleeping sickness. There are a large number of varieties of this organism which infect man and the lower animals. The **trypanosoma gambiense** can be demonstrated in the majority of cases. It is described as varying in length from 20 to 25 microns, by about 2 to 2.5 microns in width. It is distinguished by a single flagellum which extends through almost the entire length of the organism, and extends beyond its anterior end. When alive it has a slow, spiral undulating movement. (See page 106.)

LEISHMAN-DONOVAN BODIES.

These bodies were first found in the spleen of patients who were apparently suffering from an irregular form of malarial fever. The body is small or elliptical, from 2 to 3 microns in diameter, containing chromatin. The bodies occur either free in the plasma or imbedded with others in a matrix or zoöglia, often as many as a dozen being in one cluster. They are believed not to occupy the bodies of the red blood-cells. They are not demonstrable in the peripheral blood.

Staining. — Wright's modification of the Romanowsky stain: the chromatin dark, the cell-body blue, and the zoöglia a fainter mauve.

VIII.

ANIMAL PARASITES.

THE ANIMAL PARASITES OF MAN.

By "animal parasites of man" is meant animal organisms which live temporarily or permanently on or in the human body, and which receive their nourishment therefrom.

Temporary parasites include the ectoparasites (epizoa). These may inhabit the skin, conjunctival sac, the mouth, the nose, and the accessory sinuses. A familiar example of this class is the sarcoptes scabiei or itch mite.

Most of the **permanent** or **stationary** parasites are found in the internal organs, and belong to the class of entoparasites or entozoa. Many of these parasites, such as the teniæ, ascarides, and ankylostoma, inhabit man only when mature; others, of which the echinococcus is an example, inhabit man only during a certain period of their existence. Thus man may be either the actual or only the intermediate host. Again, for many parasites, such as the tenia solium and the tenia saginata, man is the only host. Finally, man may also become the host for parasites which, as a rule, select some other animal. Thus the cysticercus cellulosæ, common to the pig and the cat, may occasionally be found in man.

In this section the classification of Max Braun[1] has been adopted, and the majority of the descriptions of the parasites which follow have been abstracted from this work.

No attempt has been made to include the rarer forms of parasites, as these are considered to be beyond the scope of this work.

[1] English Translation of "The Animal Parasites of Man." (Wood & Co., 1906.)

CLASSIFICATION OF THE MORE COMMON ANIMAL PARASITES OF MAN.

A. Protozoa.

Class I. Rhizopodia.—Ameba coli *(Loesch)*.

Class II. Flagellata (Mastigophora).

(a) *Trichomonas:*

1. Trichomonas vaginalis.
2. Trichomonas intestinalis.
3. Trichomonas pulmonalis.

(b) *Circomonades:*

1. Lamblia intestinalis.
2. Tripanosoma.

Class III. Sporozoa.

Coccidia:

1. Coccidium perforans or hominis.
2. Hemosporidia.

Class IV. Infusoria.—Balantidium coli or paramecium coli.

B. Platyhelminthes *(Flat worms)*.

Class I. Trematoda *(Rud)*.

1. Fasciola hepaticum *syn.* distomum hepaticum.
2. Distomum pulmonale *syn.* distomum vestermani.
3. Distomum lanceolatum *syn.* dicrocelium lanceolatum.
4. Distomum hematobium *syn.* bilharzia, *syn.* schistosomum hematobium.

Class II. *(Rud.)*

(a) *Bothriocephaloidia:*

1. Bothriocephalus latus. *syn.* tenia lata.

(b) *Teniidæ:*

1. Tenia nana.
2. Tenia lanceolata.
3. Tenia solium.
4. Cysticercus acanthotrias.
5. Tenia saginata or mediocanellata.
6. Tenia echinococcus.

C. Nematoda *(Thread worms).*

1. Strongyloides (rhabdonema strongyloides) *syn.* anguillula intestinalis et stercoralis.
2. Filaria sanguinis hominis.
3. Trichocephalus dispar *(whip worm).*
4. Trichina spiralis.
5. Ankylostoma duodenale.
6. Uncinaria Americana.
7. Ascaris lumbricoides.
8. Oxyuris vermicularis.

A. PROTOZOA.

The protozoa is a microscopic living organism. It is monocellular and represents the lowest form of animal life. The substance of the body consists of a finely granular, contractile protoplasm, which may be mono- or poly- nuclear. The viscid hyaline entosarc is capable of motion by expansion and contraction, or by the extension and retraction of pseudopodia, cilia or flagella. Propagation takes place by segmentation or gemmination.

CLASS I. RHIZOPODIA.

The ameba coli produces the well-known amebic dysentery. The ameba in man is, however, not confined to the intestines, but has been found in the pus of liver abscesses, in pleuritic and peritoneal exudates, in the mucous membranes, and in tumors of the urinary bladder.

Characteristics.—This organism is an ameboid body measuring from 20 to 30 microns in diameter. It is composed structurally of a clear protoplasmic outer portion, ectosarc, and a finely or coarsely granular central portion, entosarc, which usually shows a number of clear vacuoles and one or more nuclei (Plate I, a). When living it shows active ameboid movements which are greatly increased if the organism is kept warm. In the living state the cell frequently includes foreign bodies, such as bacteria, pigment granules, and fragments of blood-corpuscles and other cells.

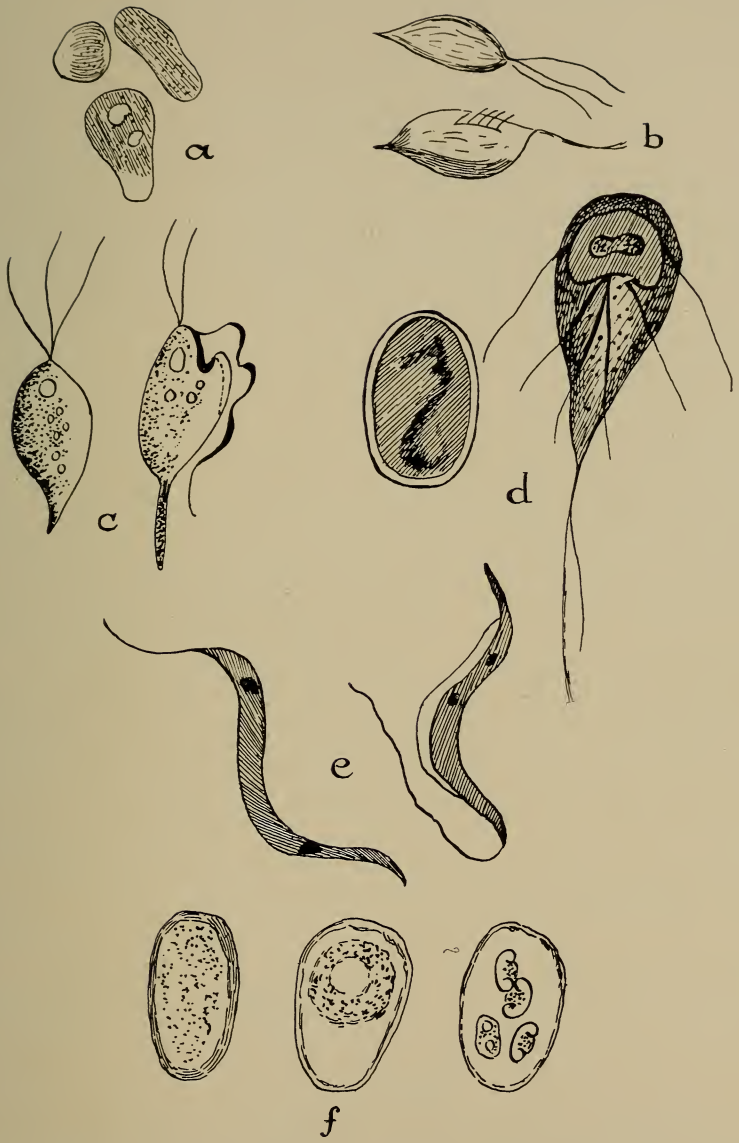
Locomotion is accomplished by irregular extension and retraction of pseudopods, which are thrown out from the periphery. These pseudopods are at first composed of the clear outer portion, which, as the projection gradually increases, includes the central granular zone. When surrounded by unfavorable environment, the organism undergoes a form of change known as the *encysted state.* In this state the body becomes spherical, and the outer wall thickened, while the division of the cell into two portions is lost, the whole becoming uniformly granular.

Method of Examination.—The fecal discharges should be caught in a warm receptacle and immediately transferred to the laboratory for examination. If it is necessary to keep the specimen for a short time, this may be accomplished by immediately placing the specimen in a thermostat at body temperature, where it should remain until transferred to the warm stage of the microscope. Preservation of specimens for more than a few hours is unsatisfactory, because even under the favorable circumstances of heat and moisture obtaining in the thermostat, motility is rapidly lost, and at the expiration of twenty-four or thirty-six hours the organisms are no longer discoverable.

The Warm Stage.—A convenient method of maintaining a warm stage for examination and study of this organism, is as follows: A flat strip of copper or of brass, three inches wide by six or eight inches long, is perforated by a half or three-quarter inch aperture, situated in the center of one end at a distance of about one inch from the free margin. The stage of the microscope should be covered with several thicknesses of asbestos paper or felt, upon which the metal sheet is clamped so that the openings in the stage and in the copper-strip coincide. A Bunsen burner or alcohol lamp is adjusted under the outer extremity of the strip, so that when the metal has attained its maximum heat the portion immediately surrounding the aperture is maintained at about body-temperature. A specimen placed upon a slide, in position for examination, will maintain the motility of the ameba for a number of hours.

It will be found convenient to examine the spread, first by the low power, then, after locating a suitable portion of the slide, the higher objective may be swung in and the organisms studied in detail. The important aids to successful search are

PLATE I.



INTESTINAL PARASITES OF MAN. (*Redrawn from Max Braun*)

a, Amebae coli. *b*, Trichomonas vaginalis. *c*, Trichomonas intestinalis. *d*, Lamblia intestinalis. *e*, Tripanosomes. *f*, Coccidium hominis.

a thin and uniform spread of liquid feces, and a low, slightly oblique illumination.

CLASS II. FLAGELLATA.

a. Trichomonides.

1. Trichomonas Vaginalis (see Plate I, b).—The form of the body is very variable, elongated, fusiform or bean-shaped. It is ameboid. The length varies between .015 to .025 millimeters in length, by .007 to .012 in breadth. The posterior extremity is drawn out into a point and is about one-half the length of the remainder of the body. The cuticle is very thin and the body-substance finely granular. At the anterior extremity there are three or four flagella which are of equal length, and which are firmly united at their base, but which easily fall off. There is an undulating membrane which moves spirally across the body, arising from the base of insertion of the flagella and terminating at the base of the caudal process. The nucleus is vesicular, elongated, and situated in the anterior extremity. Propagation occurs by division.

2. Trichomonas Intestinalis (see Plate I, c).—Some authors believe this organism to be identical with the trichomonas vaginalis. It is described as being .01 to .015 millimeter in length, the posterior extremity terminates in a point with a row of cilia. These commence at the anterior extremity and extend over the body.

This organism has been found in the urethra of man, the vagina, the large and small intestines of both healthy and sick persons, in the stomach and in the oral cavity.

3. Trichomonas Pulmonale. In all probability this is identical with the preceding.

b. Cercomonides.

1. Cercomonas Intestinalis or Lamblia Intestinalis (see Plate I, d).—Length, .01 to .020 millimeter, and width, .005 to .012. The flagella are of about equal length (.009 to .014 millimeter). The body is finely granular, and has a very thin cuticle which does not entirely prevent changes in the form of the body. The very motile tail-like appendages in the frontal

plane are flattened; the excavation at the anterior extremity which is directed obliquely forward and with its border projecting backward, no doubt corresponds to the peristome, and serves as a clinging organ. The first pair of flagella (anterior flagella) arise from the anterior edge of the peristome; the second and third pairs (lateral and median) from the posterior edge, whereas the tail flagella are inserted at the posterior end of the tail. In life the anterior and lateral flagella sway only with the part projecting from the body; the median and tail flagella are quite free. The anterior flagella appear to be connected with the nucleus. The nucleus is dumb-bell shaped and has a nucleolus in each half, and lies anteriorly in the part of the body which is excavated. This organism has *encysted stages*. These *cysts* are oval and measure .01 by .007 millimeter. They are surrounded by a fairly thick hyaline layer through which the outline of the creatures can sometimes be seen quite distinctly.

2. Tripanosoma (see Plate I, e).—The tripanosomes, as usually seen in the blood of man, are elongated, unicellular, microscopic bodies, provided with a kind of fin-fold. The undulating membrane which runs along the dorsal edge of the body forms frill-like folds and terminates in a free whip-like filament (flagellum), which may vary greatly in length.

Stained specimens show a large nucleus, usually situated at about the middle of the body, and a much smaller chromatin mass placed more or less near one pole. This chromatin mass is called the centrosome. The extremity of the body which encloses this is its anterior end. This organism varies greatly in length and shape in different stages of its development; it may be pointed or obtuse. The posterior extremity always tapers and is continued into the free flagellum. Sometimes the body of the stained specimen shows many darkly stained granules called chromatophores. (See page 100.)

CLASS III. SPOROZOA.

Coccidia.

1. Coccidium Hominis or Perforans.—This organism is oval. The fertilized sporont stage is the oöcyte (see Plate I, f), and measures .024 to .035 by .002 to .014 millimeter. It is surrounded by an integument with a double outline which has an

opening at the pointed pole. The plasm, which is somewhat coarsely granular, entirely fills the integument or is gathered together in a rounded central mass. The coccidia are evacuated from the bowel in this stage, and sporulate in the open within two or three weeks. The fully developed *spores* are of a broad fusiform shape and measure .012 to .015 millimeter in length by .007 millimeter broad. They each contain two *sporozoites,* broad at one end and pointed at the other, forming a bent dumb-bell shaped body. The chromatin mass is called the *centrosome,* and the extremity of the body which encloses this, is the anterior extremity.

A granular residual body lies in the concavity. This organism is found in the intestinal tract, where it may give rise to violent auto-infection and chronic diarrhea.

2. Hemospoidiæ.—These organisms are the cause of malaria and have been described in another section (see page 96).

CLASS IV. INFUSORIA.

Balantidium Coli or Paramecium Coli.—The *body* is oval, .06 to 1.0 millimeter in length, by .05 to .07 millimeter in width. The peristome is funnel-shaped or contracted, the anterior end being either blunt or pointed. The ecto- and entosarc are distinctly separate, the latter granular, and containing drops of fat, mucus, starch granules, bacteria, and occasionally leukocytes and erythrocytes. There are usually two contractile vacuoles. The anus opens at the posterior extremity, and the organism contains a macro- and a micro-nucleus. It occurs in the large intestines of man.

B. PLATYHELMINTHES (Flat Worms).

CLASS I. TREMATODES.

1. Distomum Hepaticum *(Liver fluke).*—Length, 20 to 30 millimeters; breadth 8 to 13 millimeters. The *head-cone* is 4 to 5 millimeters long, and sharply demarcated from the posterior part of the body. *Spines* in alternating transverse rows, and extending on the ventral surface to the posterior border of the testes, and on the dorsal surface not quite so far. The spines are smaller on the head-cone than on the posterior part

of the body, where they are discernible by the naked eye. The *suckers* are hemispherical and near each other. The oral sucker is about 1 millimeter, and the ventral about 1.6 millimeter in diameter. The *pharynx*, which includes almost the entire esophagus, measures .7 millimeter in length and .4 millimeter in breadth. The intestine bifurcates in the head-cone, and the branches are furnished with blunt sacs directed outwardly. The *ovary* is ramified and is situated in front of the transverse vitello duct. The shell-glands lie near the ovary and are in the median line.

The *ova* are yellowish-brown, oval, with cap-like lid. They measure .130 to .145 by .07 to .09 millimeter. The average size is .132 by .08. The liver fluke is an inhabitant of the bile-ducts of man.

2. Distomum Pulmonale or Distomum Westermanni.—The *body* is of a faint, reddish-brown color and plump oval shape, with the ventral surface a little flattened. This organism measures 8 to 10 millimeters in length by 4 to 6 millimeters in thickness. It possesses *two suckers* of equal size (.75 millimeter).

The *eggs* are oval brownish-yellow, with a fairly thin shell and measure .0875 to .1025 millimeter in length, by .052 to .075 millimeter in breadth, the average being .0935 by .057 millimeter. Their location is usually in the lung, but they may enter the blood-vessels and be carried to another part of the body.

3. Distomum Lanceolatum or Dicrocelium Lanceolatum.—In the fresh condition this is a yellowish-red organism, the *body* is flat, almost translucent, with a conical neck at the level of the ventral sucker. This point is marked by a shallow constriction. The length and breadth vary according to the amount of contraction, being usually 8 to 11 millimeters by 1.5 to 2.0 millimeters. The *suckers* are about one-fifth of the body-length distant from each other, and of about equal size (.23 by .25 millimeter).

The *eggs* are oval with a sharply defined operculum at the pointed pole. They measure .030 by .011 millimeter, and occur in the feces. The parasites reside in the liver.

4. Distomum Hematobium or Bilharzia.—The *male* is

whitish and measures 12 to 14 millimeters in length, but is already mature when 4 millimeters long. The anterior end is 6 millimeters or a little more in length; the suckers are situated near each other; the oral sucker is infundibuliform; the dorsal lip being a little longer than the ventral. The ventral sucker is slightly the larger, and is pedunculated. A little behind the ventral sucker the body broadens to a width of 1 millimeter, decreasing, however, in thickness. The lateral edges curl ventrally, so that the posterior part of the body is almost cylindrical. The posterior end is somewhat attenuated, and the dorsal part of the posterior extremity of the body is covered with spinous papilli.

The *females* are filiform, about 30 millimeters in length, pointed at each end and measuring .25 millimeter in diameter. The color of the body varies according to the contents of the intestine (posteriorly they are dark-brown or black). The cuticle is smooth except in the suckers, where there are very delicate spines, and at the tail end where there are larger spines. The anterior part of the body measures from .2 to .3 millimeter in length.

The *eggs* are fusiform and much dilated in the middle. They have no lid and are provided with a terminal spine at the posterior extremity. The eggs *measure* 0.12 to 1.12 millimeters in length, by .05 to .073 millimeter in breadth. They are yellowish in color, slightly transparent, and provided with a thin shell. The spine may sometimes be absent. The eggs apparently vary greatly in size. The organism lives in the portal vein and its branches.

CLASS II.

a. Bothriocephaloidia.

1. Bothriocephalis Latus or Tenia Lata. — *Length* from 2 to 9 or more millimeters. Color, yellowish gray; after lying in water lateral areas become brownish and the rosette of the uterus brown. The *head* is almond-shaped, 2 to 3 millimeters in length. Its dorso-ventral axis is longer than its transverse diameter; the head is therefore generally flat, concealing the suctorial grooves at the borders. The neck varies in length according to the degree of contraction, and is very thin. There

are from 3000 to 4500 *proglottides*. Their breadth is usually greater than their length, but in the posterior third of the body they are almost square, while among the very oldest some may be longer than they are broad.

The *eggs* are large with brownish shells and small lids. They measure .068 to .071 by .045 millimeter. The proglottides near the posterior extremity of the worm are frequently eggless.

b. Teniidae.

1. Tenia Nana.—The worm is 10 to 15 millimeters in length, and .5 to .7 millimeter in breadth. The *head* is globular and is from .25 to .30 millimeter in diameters. The rostellum has a simple crown consisting of 24 to 30 hooks, which are only .014 to .018 millimeter in length. The neck is moderately long. The *proglottides* are very narrow, about 150 in number, .4 to .9 millimeter in breadth, by .014 to .030 millimeter in length.

The *eggs* are globular or oval, and measure .030 to .048 millimeter. The oncospheres measure .016 to .019 millimeter in diameter.

The worm lives in the intestines; the ova, and proglottides are found in the feces.

2. Tenia Lanceolata or Hymenolepis Lanceolata.—The parasite measures 30 to 130 millimeters in length, and 5 to 18 millimeters in breadth. The *head* is globular and very small, the rostellum is cylindrical with a crown composed of eight hooks (0.031 to 0.035 millimeter in length). The neck is very short. The segments increase gradually in breadth, but vary little in length.

The *ova* have three envelopes and are oval, measuring 0.050 by 0.035 millimeter. The external envelope is membranous and much wrinkled, the middle one is thick, and the internal one very thin.

3. Tenia Solium or Tenia Vulgaris. — The average length of the entire tapeworm is about 2 to 3 meters, but may be more. The *head* is globular, 0.6 to 1.0 millimeter in diameter. The rostellum is provided with a double row of hooks, twenty-two to thirty-two in number; large and small hooks alternate regularly. The length of the largest hooks is 0.16 to

0.18 millimeter, of the small ones 0.11 to 0.14 millimeter. The average number of *proglottides* is 800 to 900; they increase very gradually in size. At about 1 millimeter behind the head they are square and have the generative organs fully developed. Segments sufficiently mature for detachment measure 10 to 12 millimeters in length, by 5 to 6 millimeters in breadth. The fully devloped uterus consists of a median trunk with seven to ten lateral branches on each side, some of which are again ramified.

The *eggs* are oval, the egg-shell very thin and delicate. The *embryonal shell* is very thick with radial stripes; it is of a pale-yellow color, globular, and measures 0.031 to 0.036 millimeter in diameter. The *oncospheres* with six hooks are likewise globular, and measure 0.02 millimeter in diameter.

4. Cysticercus Acanthotrias.—This resembles the *cysticercus cellulose* in form and size, but carries on the rostellum a triple crown each consisting of fourteen to sixteen hooks which differ from the hooks of the cysticercus cellulose or of the tenia solium by the greater length of the posterior root process and the more slender form of the hooks. The large hooks measure 0.153 to 0.196 millimeter, the medium-size hooks 0.114 to 0.14, and the small ones 0.063 to 0.07.

5. Tenia Saginata or Tenia Mediocanellata. — The length of the entire worm averages 4 to 8 to 10 meters and more, even up to 36 meters. The *head* is cuboid in shape, 1.5 to 2 millimeters in diameter. The suckers are hemispherical (0.8 millimeter), and are frequently pigmented. There is a sucker-like organ in place of the rostellum, and this is also frequently pigmented. The neck is moderately long and about half the breadth of the head. The *proglottides* average more than 1000, and gradually increase in size from the head backward. The detached mature segments are exactly like pumpkin-seeds—they are about 16 to 20 millimeters long, by 4 to 7 millimeters broad. There are twenty to thirty-five lateral branches at each side of the uterus, and these often again ramify.

The *eggs* are more or less globular, the egg-shell frequently remains intact, and carries one or two filaments. The *embryonal shell* is thick, radially striated, transparent and oval. It is 0.3 to 0.4 millimeter in length, by 0.02 to 0.03 millimeter in breadth.

This worm in the adult condition dwells exclusively in the intestinal canal of man. The corresponding *cysticercus* occurs in the ox and steer.

6. Tenia Echinococcus.—This worm measures 2.5 to 5 or 6 millimeters in length; the *head* is 0.3 millimeter in breadth, and has a double row of twenty-eight to fifty hooklets on the rostellum. The size and form of these hooklets vary. The larger ones are 0.040 to 0.045 millimeter in length, the smaller ones are 0.030 to 0.038 millimeter. The suckers measure 0.13 millimeter in diameter. The neck is short, behind which there are only three or four segments, the posterior of which is about 2 millimeters in length and 0.6 millimeter in breadth. The ovary is horse-shoe shaped, with the concavity directed backward. The median trunk of the uterus is dilated when filled with eggs, and instead of lateral branches has lateral protuberances. It is not uncommon for the *eggs* to form local heaps. The *embryonal shell* is moderately thin with radiating fibers, is almost globular, and measures 0.030 to 0.036 millimeter in diameter.

The mature parasite lives in the small intestine of the domestic dog and the wolf, and from them, the dog chiefly, they are transmitted to man.

Diagnosis of Cestodes.—The microscopic examination of the feces should never be neglected when the presence of tapeworm is suspected. Often by careful, frequently repeated examinations, insistent symptoms, referable to the digestive tract, the nervous system or the general nutrition, may be cleared up by the finding of segments or ova in the feces.

As the uterus of the tenia has no exit, the eggs can only find egress when the mature proglottide is injured. In the case of the tenia saginata the discharge of eggs is almost the rule. The proglottides when discharged are usually without eggs. The eggs of the solium and the saginata are only distinguished by their size.

In examining the stools for evidences of tapeworms, one must be careful not to confound remnants of undigested food, mucous casts, and shreds of tendon with the proglottides. The proglottides, after being soaked in water, assume their characteristic form. As a rule the microscopic determination of ova is a more certain means of diagnosis than the macroscopic segments.

To determine from the shape of the proglottide which variety of worm is present, it is advisable to fix the segment between two glass slides. The proglottide of the tenia solium is more delicate and more transparent than the tougher segments of the tenia saginata. In the former the branching uterus is more plump, and the number of lateral twigs are from seven to ten, while the uterus of the tenia saginata shows from twenty to thirty or more.

C. NEMATODES (Thread Worms).

Strongyloides Intestinalis or Anguillula Intestinalis et Stercoralis.—1. (a) The *parasitical generation (anguillula intestinalis)* measures 2.3 millimeters in length by 0.034 millimeter in breadth. The cuticle is finely transversely striated. The mouth is surrounded by four lips, the esophagus is almost cylindrical and is a quarter the length of the body. The anus opens just in front of the pointed posterior extremity.

The *eggs* measure 0.050 to 0.58 millimeter in length, and 0.030 to 0.034 in breadth.

(b) The *free-living generation (anguillula stercoralis)* is sexually differentiated. The body of the *male* is cylindrical, smooth, somewhat more slender at the anterior extremity, and pointed at the tail end. The mouth has four lips and the esophagus a double dilatation. The males measure 0.7 by 0.035 millimeter, and carry the posterior extremity curled up. The two spicules are small and much curved. The *females* measure 1.0 millimeter in length or a little more, 0.05 millimeter in breadth. The tail end is straight and pointed. The yellowish thin-shelled *ova* measure 0.07 millimeter in length by 0.045 millimeter in breadth.

2. Filaria Sanguinis Hominis *(filaria bancrofti or filaria nocturna).*—The *male* is colorless and measures 40 millimeters in length and 0.1 millimeter in diameter. The *cephalic extremity* is a little thickened, the posterior extremity is bent and rounded, but is not twisted cork-screw like. The anal orifice opens 0.138 millimeter in front of the posterior border. The *female* is brownish, 7.8 to 8.0 millimeters in length and 0.21 to 0.28 millimeter in breadth. The cephalic and caudal extremi-

ties are rounded. Almost the entire body is occupied by the two uteri, from which the larvæ emerge early. The length of the *larvæ* average 0.13 to 0.3 millimeter, their breadth 0.007 to 0.07 millimeter. They are surrounded by a delicate protective investing membrane which is not quite close to them.

The lymphatic vessels in various parts of the body are doubtless the normal habitat of the adult worms, but these have also been found in the left ventrice of the heart. The young ova, by means of the lymph-stream, reach the blood and are distributed with it through the body. They also pass through the vessel walls and may be found in the fluid of the glands of the body. The larvæ are first found in infected patients only in specimens of blood that have been taken after sunset. Their number increases considerably until after midnight, and after that time begin to diminish. From mid-day until evening no filariæ are found in the peripheral blood.

3. Trichocephalus Dispar or Ascaris Trichiura.—The *male* measures 40 to 45 millimeters in length, the spiculum is 2.5 millimeters long and lies within a retractile pouch beset with spines. The *female* measures 45 to 50 millimeters in length, of which two-fifths appertain to the posterior part of the body. The ova are barrel-shaped and have a thick, brown shell which is perforated at the poles. Each opening is closed with a light-colored plug. The *eggs* measure 0.05 to 0.054 millimeter in length and 0.023 millimeter in breadth. They are deposited before segmentation. This worm usually lives in the cecum of human beings, and is occasionally found in the vermiform appendix, in the colon, and exceptionally in the small intestine. Usually only a few are present, and they do not cause any particular disturbance.

The *development of the eggs* is completed in water or in moist soil, and occupies a longer or shorter period according to the season. The eggs and larvæ possess great powers of resistance, and have been known to remain as long as five years in the egg-shell without losing their vitality.

4. Trichina Spiralis.—The *male* measures 1.4 to 1.6 millimeters in length and 0.04 millimeter in diameter. The anterior part of the body is narrowed, the orifice of the cloaca is terminal and lies between the two caudal appendages; behind

there are four papillæ. The *female* measures 3 to 4 millimeters in length and 0.06 millimeter in diameter; the anus is terminal. Trichina spiralis occupies in its adult stage the small intestines of men and of various mammals, including the domestic rat, domestic pig and domestic dog.

History of Development of Trichina Spiralis.—Shortly after entering the intestines the encysted trichinæ escape from their capsules, and then enter the duodenum and jejenum where they become adult. During this period they do not greatly increase in size. The males grow from 0.8 to 1.0 millimeter, the females from 1.5 to 1.8 millimeters. Soon after copulation, which takes place in the course of two days, the males die off, and the females, which soon attain the length of 3.0 to 3.5 millimeters, either bore more or less deeply into the villi or penetrate the mucous membrane and enter the lymphatic spaces. Here they deposit their young which, according to Leuckart, average at least 1500. The migrations are mostly passive, the larvæ being carried along by the lymph-stream or by the circulating blood. The young brood is distributed throughout the entire body, but the conditions necessary to its further development, are found only in the transversely striated muscle. On the ninth or tenth day after infection the first trichinæ have reached their destination, but further invasions are constantly taking place. Two or three weeks after infection the spirally rolled up trichinæ have grown to 0.8 to 1.0 millimeter, and in their vicinity the muscle fibers are swollen. The capsule is formed by the inflamed connective tissue producing the cystic membrane. The *cysts* are lemon-shaped and usually lie with their longitudinal axis in the direction of the muscle fibers. On an average they measure 0.4 millimeter in length by 0.25 millimeter in breadth.

5. Ankylostoma Duodenale or Uncinaria Duodenale.—The body is cylindrical, attenuated anteriorly, and of a slightly reddish color. In the oral cavity on the ventral surface, close behind the orifice, are four hook-like teeth directed backward; on the dorsal surface there are two teeth directed forward. The *males* measure 8 to 10 millimeters in length, and 0.4 to 0.5 millimeter in breadth. The bursa has two large lateral and one small dorsal alar processes. The *females* measure 12 to

18 millimeters in length, and the caudal extremity has a small spine. The *eggs* are elliptical and have thin shells; they measure 0.032 to 0.045 millimeter in breadth, and 0.055 to 0.065 millimeter in length.

The ankylostoma duodenale lives in the duodenum, and may rarely be found in the first part of the jejunum.

6. Uncinaria Americana can readily be distinguished from the preceding worm. It is shorter and more slender. The *male* worm measures from 7 to 9 millimeters in length by 0.3 to 0.35 in diameter: the *female* 9 to 11 millimeters in length by 0.4 to 0.45 millimeter in diameter. The buccal capsule is much smaller, and presents an irregular border; instead of four ventral hook-like teeth, it is provided with a vertical pair of prominent semilunar plates similar to those of a dog hook-worm. The pair of dorsal teeth is likewise represented by a pair of slightly developed chitinous plates of the same nature.

The *eggs* are larger than in U. Duodenale; they measure 64 to 75 micromillimeters by 36 by 40 micromillimeters in breadth. So far this worm has been found only in man; its anatomical *habitat* is the small intestine.

7. Ascaris Lumbricoides.—The coloring in the fresh stage is reddish- or grayish-yellow. The body is of an elongated spindle shape and the dorsal oral papillæ carries two papillæ of sense and the two ventral oral papillæ are papillæ of sense. The *male* measures from 15 to 25 centimeters in length and about 2 millimeters in breadth. The posterior extremity is conical and bent ventrically into a hook. The *spicules* measure 2 millimeters in length and are curved and broadened at their free ends. On each side of the orifice of the cloaca are seventy to seventy-five papillæ, of which seven pairs are post-anal.

The *female* measures 20 to 40 centimeters in length and about 5 millimeters in diameter, the posterior extremity is conical and straight. The vulva is at the border between the middle and posterior thirds of the body, from which the two uterine tubes pass straight to the posterior end of the body. The convoluted ovaries measure ten times the length of the body.

The *ova* are elliptical with a thick transparent shell and an external coating of albumin which forms protuberances. The ova measure 0.05 to 0.07 millimeters in length and 0.04 to 0.05

millimeters in breadth; they are deposited before segmentation. The albuminous coating is stained yellow by the coloring matter of the feces.

This worm is one of the most frequent parasites of man, and is distributed over all parts of the world.

8. Oxyuris Vermicularis or Ascaris Vermicularis.—Color, white; the attenuated cuticle forms swellings at the anterior end which extend some distance back along the middle of the ventral and dorsal surfaces. There are three small retractile labial papillæ around the mouth. The *male* measures 3 to 5 millimeters in length, and shortens on death. The posterior extremity of the body is rolled ventrally and presents papillæ. The *female* is 10 millimeters in length and 0.6 millimeter in diameter. The anus is about 2 millimeters in front of the tip of the tail; the vulva is in the posterior third of the body. The *eggs* are oval, thin-shelled, and measure 0.05 by 0.02 millimeter. They are deposited with embryos already developed, and are seldom found in the feces.

IX.

DETERMINATION OF THE FUNCTIONS OF THE STOMACH.

THE GASTRIC CONTENTS.

The significance of the term gastric contents, in the following pages, is taken to mean the material found in the stomach and extracted by the gastric tube at the expiration of a fixed and definite period after the ingestion of a test-breakfast.[1]

THE VOMITUS.

It is not good practice to utilize vomitus for the purpose of chemical analysis. Such material is usually of very uncertain composition, being contaminated with mucus from the upper part of the tract; further, the amount or composition of the food previously ingested is an unknown factor, affecting materially the quantitative and qualitative findings. However, it may be found of decided advantage to test all vomited matter for the presence or absence of acidity and free hydrochloric acid. In the presence of suspected cancer the vomited material may be searched for necrotic tissue shreds or sarcinæ.

METHODS OF OBTAINING SPECIMEN FOR EXAMINATION.

The usual apparatus employed to remove the test-meal comprises the well-known gastric tube of soft red rubber, fitted at one end with a soft rubber funnel, and containing near this a bulbous expansion without valves. A recent modification of and improvement over this is the large bulb devised by Ewald. This bulb is sufficiently large to contain the total quantity of

[1] The Ewald test-breakfast consists of an ordinary roll weighing about 35 grams, and 300 centimeters of water or weak tea without milk or sugar.

material removed, thus overcoming in a measure the difficulties of the smaller bulb. These two methods are usually successful in obtaining the desired material for examination, but are difficult to manage and possess a decided disadvantage in that they

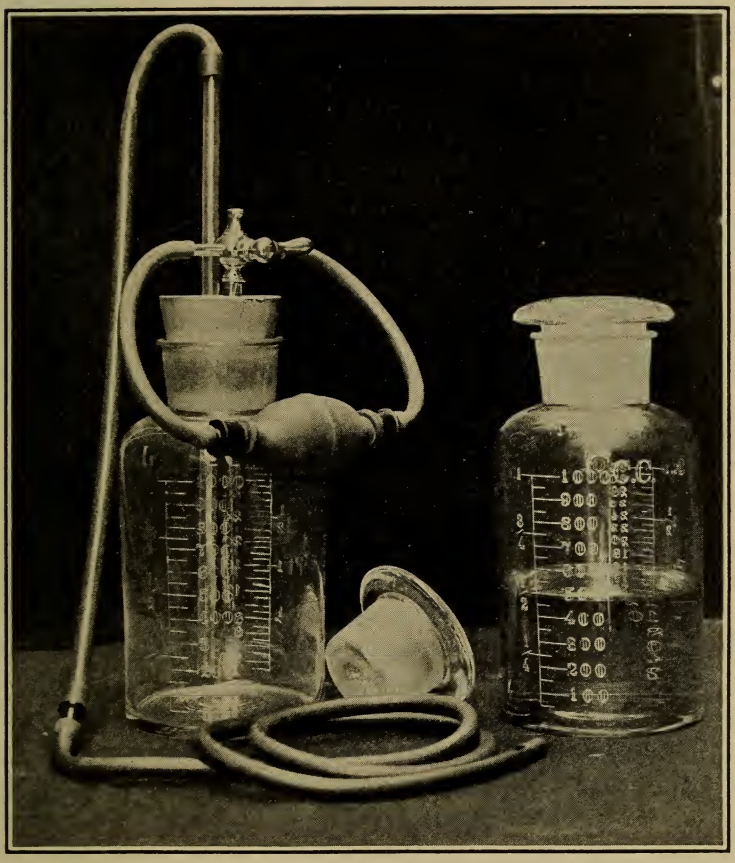


FIG. 15.—COMPLETE OUTFIT FOR GASTRIC TEST-MEAL REMOVAL, LAVAGE AND INFLATION.

do not provide for the important procedure of lavage and inflation.

Also, tubes of irregular caliber are difficult to cleanse, and unless great care is exercised in this direction, they may become a source of contamination, if not carriers of infection.

Some years ago Dr. Judson Daland adopted for this purpose two large open-mouth bottles and a double-action Davidson bulb; these are used in conjunction with the plain gastric tube. With this arrangement, aided by an assistant, it is possible to rapidly and cleanly obtain a sample of gastric contents (in considerably less than a minute), and to follow immediately with lavage and inflation if desired.

To those who have used this method with uniform success in both private and hospital practice, its advantage is evident, the only drawback being the almost absolute necessity for trained assistance, this being occasioned by the complicated nature of the apparatus, which requires a second pair of hands to manipulate the bottle and its connections during the passage of the tube. To overcome this drawback the author has devised a reversing valve (see Fig. 15). With this valve it is unnecessary to make any change in the tube connections when once the apparatus is set up. The perfected device has been worked out with great care, having in mind the necessity of a simple mechanism capable of being cleansed and kept in repair without difficulty.

PRELIMINARY PREPARATION OF THE PATIENT.

The preliminaries leading up to the extraction of the test-meal should be as nearly uniform in every case as possible. By adopting a definite routine and adhering to it we eliminate, in a great measure, the errors which would otherwise creep in and lesson the value of the findings. The adoption of the following rules will enable the examiner to obtain a series of reports in one case or in groups of cases of far greater value than could be obtained by an irregular technic:—

First.—It is advisable that all medication should be withheld until after the gastric analysis has been made. If this is impossible, then a period of days should elapse before making the test, during which all treatment is stopped.

Second.—The test-meal should be given on an empty stomach; either one which has been emptied by fasting or by previous lavage.

Third. — The volume and composition of the test-meal should be uniform. The particular meal employed to be determined by the operator according to his preference.

Fourth.—The removal of the meal should (depending on its composition) be accurately timed; this time to be measured from the beginning and not from the end of the meal.

Fifth.—The test-meal should be removed without dilution if possible. If dilution should be necessary to accomplish removal, the amount of water used should be definitely known, and the total withdrawn must exceed the amount used. If this rule is not observed, all investigations of a quantitative nature are valueless.

Sixth.—The tube should remain within the esophagus only long enough to allow time to compress the bulb. If much time elapses after introduction before removal of the contents, the hypersecretion of mucus occasioned by the presence of the tube will alter the composition of the sample and possibly result in erroneous conclusions.

A tube of relatively large diameter is to be preferred, being easier of insertion, less uncomfortable to the patient, and insuring greater success in obtaining a sample. A suitable tube should measure between three-eighths and one-half inch outside diameter, and should be provided with both a terminal and lateral opening in the gastric end.

A lubricant for the tip of the tube is unnecessary. The contact of the tube with the patient's pharynx immediately exciting sufficient flow of mucus for this purpose. This is preferable to either nauseating oil or hygroscopic glycerine. In case of great reflex excitability of the pharyngeal constructors, this region may be first sprayed with a dilute solution of cocaine.

Modified Ewald Breakfast.—Of the test-meals employed, one of generally useful composition includes the white of two eggs poached or soft boiled, without yelks and without seasoning; two pieces of toast without butter (the slightest trace of butter or fat will cause lactic and butyric acid fermentation), and a cup of tea without milk or sugar.

This meal should be removed at the expiration of one hour when, under ordinary circumstances, there will be recovered between 30 and 90 cubic centimeters.

The component parts of the apparatus are as follows:—

A plain gastric tube without bulb or funnel.

A double-action Davidson hand-bulb.

A large rubber stopper having two perforations.

Two wide-mouth bottles containing more than a liter each, and graduated in cubic centimeters.[2]

A short length of large glass-tubing and some one-quarter inch rubber-tubing.

The reversing valve.

Technic of Removal.—Fill one graduated bottle to the 500 cubic centimeter mark with warm, sterile water. Fit the double perforated stopper with the glass tube and the reversing valve. Place the stopper firmly in the empty bottle and attach the gastric tube to the glass tube, and the two ends of the double action bulb to the two horizontal tubes of the valve. Finally, ascertain the direction of the air-current through the valve by making a few pressures on the bulb. Set the valve to make negative pressure within the bottle.

The tube should now be passed, with the patient preferably in the sitting posture. As soon as the tube enters the cardiac orifice, a few quick pressures are made on the bulb. This develops a slight degree of negative pressure within the bottle, when the gastric contents will immediately flow into the bottle. Sudden stopping of the flow from occlusion of the tube by particles of food or mucus, may immediately be removed by momentarily reversing the lever. This will cause a small portion of the gastric contents to return through the tube, effectually washing out the obstruction. This simple maneuver may be repeated as frequently as necessary to obtain a sufficient specimen.

In the event of failure to obtain sufficient material by this means, the difficulty will usually be overcome by the introduction of a measured amount of water. To accomplish this the stopper with all its connections is removed from the bottle and fitted into the bottle containing the 500 cubic centimeters of warm water. The valve is set to make positive pressure within

[2] These special bottles are not necessary for practical purposes; any large, open mouth bottle of sufficient quantity, such as a quart milk bottle, may be substituted. A mark must be made at the measured 500 cubic centimeter mark on one bottle.

the bottle, and by means of the bulb about 400 cubic centimeters are run into the stomach; then by reversing the valve the whole is withdrawn. It is necessary to recover more than a total of 500 cubic centimeters if any determinations of a quantitative nature are to be made.

If it has been necessary to resort to the introduction of water to effect the removal of the test-meal, allowance must then be made in the final calculations, so that the results will represent the quantities in pure gastric contents.

For Example.—Suppose after employing 500 cubic centimeters a total of 550 cubic centimeters is recovered, of this amount only 50 cubic centimeters represent actual gastric contents, or one part in every eleven. It will be necessary then to multiply any figures obtained in the calculations of acidity by the factor eleven in order to express the results in terms of undiluted gastric contents.

Inflation of the Stomach.—Next to the *x*-ray, probably the best method of determining the size, shape, and location of the stomach is by the introduction of air. To accomplish this two methods are available. Of these, the second is greatly to be preferred for reasons to be stated later:—

1. The first consists in administering one dram of sodium bicarbonate dissolved in a little water, to be immediately followed by an equal quantity of tartaric acid, also in solution. The combination of these causes the evolution of carbon dioxide gas within the stomach, which immediately distends that organ. This method is open to serious objection, because the quantity of gas produced cannot be controlled and over-production, besides causing great discomfort if not doing actual damage, may result in hemorrhage and great cardiac embarrassment. On the other hand, sufficient gas may not be evolved to completely distend the stomach, and thus its full size and shape fail to be accurately determined. In the light of these facts it would seem best that this method be permanently abandoned for the following, which is more in accord with the principles of scientific medicine.

2. The second method of inflation is accomplished through the stomach tube by means of a Davidson bulb. This simple combination may be employed with safety and accuracy, and

even in the absence of the graduated bottles and reversing valve can be made to serve.

A greater refinement in the technic is attainable with the aid of bottles and valve above described. Their use may conveniently follow the removal of the test-meal. With this outfit in addition to inflation we may also roughly measure the cubic contents of the distended stomach, by introducing a measured quantity of air.

Technic of Inflation.—By inflation the position of the stomach may be accurately outlined. After the test-meal has been removed the patient is placed in the semi-recumbent posture, and the empty stomach is outlined as accurately as possible by auscultatory percussion. Air is then introduced into the stomach through the tube in sufficient quantity to produce a distinct change in the auscultatory percussion-note. The quantity of air required to accomplish this is quite small, not sufficient to alter the relation of the organ to surrounding viscera, as is the case when the stomach is ballooned with air. By the proper working of the valve and the bulb on the apparatus, we can change the gastric note at will, thereby being able to differentiate absolutely between gastric and colonic tympany.

By this method gastroptosis can be absolutely determined, the author having in many instances had the results confirmed by the x-ray.

Contraindications to Inflation.—These are the same as for the use of the stomach-tube itself, viz.: Myocardial degeneration, with or without endocarditis; angina pectoris, aneurism, advanced vascular degeneration, hemorrhage from any part of the body, and all diseases in which hemorrhage is likely to occur. Gastric ulcer is, of course, a contraindication, especially when hemorrhage has been noted. Another contraindication, and one which is not sufficiently emphasized, is neurasthenia and allied mental states with gastric symptoms. In these diseases the symptoms referable to the stomach are not due to organic change in that organ, but are psychic in origin. The use of the tube in these cases serves but to reinforce the idea of disease of the stomach, and renders a cure more difficult and occasionally impossible. This, of course, refers to those cases which are obviously psychic in origin. In certain cases it is not possible to

determine whether the disease is organic or not; here the tube would have to be used for diagnostic purposes. It has been suggested that the unpleasantness of the passage of the tube itself will act as a curative agent. Experience teaches that usually more harm than good will come from the use of the tube with this intent.

To Determine the Capacity of the Stomach by Inflation.—To accomplish this the apparatus employed in the preceding de-

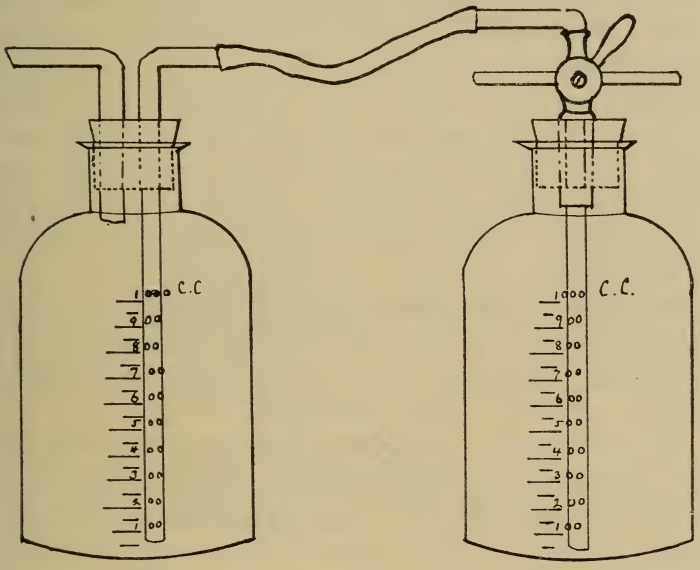


Fig. 16.—Diagrammatic Representation of Arrangement of Bottle for Measuring Cubic Contents of Stomach.

scription must be augmented by the addition of a second double-perforated rubber stopper and a second section of glass-tubing, arranged in a similar manner to the first.

The apparatus is to be set up as follows (see Fig. 16): The extremity of the gastric tube is attached to the short glass-tube of the second stopper, and a section of tubing made to join the long glass tube of each stopper. The bottle belonging to the valve and bulb is filled with water to the 1000 cubic centimeter mark, and both stoppers placed in their respective bottles. Now, with the tube in the stomach and the valve set for compression,

the water is gradually forced over into the second bottle. This in turn displaces the air, and forces it into the stomach. When the patient indicates that the stomach is full, the amount of water displaced from the first bottle will equal the amount of air forced into the stomach, and can be read in cubic centimeters from the scale on the bottle. (See diagram.) The valve should now be reversed and the air withdrawn.

Technic of Gastric Lavage.—Prepare a large pitcher of sterile water at body temperature, and a receptacle for waste. If desired normal saline or dilute alkaline water may be substituted. Place 500 cubic centimeters of the wash-solution in one bottle, and by means of the valve and bulb force a few hundred centimeters into the stomach; then, after allowing it to remain for a few moments, reverse the valve and withdraw as much as possible. Discard the returned water and repeat this process until the wash-water returns clear. Finally, a quantity of this saline or alkaline solution may be allowed to remain when the tube is withdrawn.

Indication for the Use of the Gastric Tube.—The tube is positively indicated in cases of carcinoma associated with pyloric stenosis. The result here is decided relief from distressing symptoms, and often actual prolongation of life.

In certain cases of pernicious anemia, especially in the later stages, stagnation of food gives rise to distressing symptoms, and by allowing absorption of toxins, hastens the progress of the disease.

Finally, in cases where poison has been taken into the stomach in toxic doses, the stomach tube is often of great service in affording prompt removal.

THE ACIDS OF DIGESTION.

Under conditions of health, after from ten to fifteen minutes following the ingestion of food the gastric contents are acid, due to the presence of free acids or acid salts. At this time the free acid recognized is lactic acid. Up to thirty-five or forty minutes lactic acid predominates, and only traces of HCl can be detected. Shortly after this the lactic acid disap-

pears and only HCl remains, so that at the end of one hour no lactic acid can be demonstrated.

Hydrochloric acid is actually present from the beginning, but its presence is masked by the excess of lactic acid and the HCl combined with bases. Free HCl increases with the progress of digestion until it reaches 0.15 to 0.20 per cent. after a light meal, or from 0.20 to 0.33 per cent. after an abundant meal.

CHEMICAL COMPOSITION OF THE GASTRIC JUICE
(containing water with saliva).

Water	994.40	parts.
Solids	5.60	"
Organic material	3.10	
Mineral salts	2.50	
Sodium chlorid	1.46	
Calcium chlorid	0.16	
Potassium chlorid	0.55	
Ammonium chlorid	Trace.	
Calcium phosphate	Trace.	
Magnesium phosphate	Trace.	
Iron	0.12	
Free HCl	0.20	

FREE ACIDS.

For the simple qualitative demonstration of free acids, organic and inorganic, the *congo-red* and *tropeolin papers* are convenient. The former turns dark-blue, the latter dark-brown, when moistened with a solution containing free acids, but neither of these react to acids which are combined with bases.

Detection of Free Hydrochloric Acid.—For the qualitative determination of the presence of free HCl, a number of tests are available.

Tests. — 1. Töpfer's Dimethyl-amido-azobenzol: For this test either the 0.5-per-cent. alcoholic solution of this chemical can be used, or for convenience filter-paper may be soaked in the 0.5-per-cent. solution, allowed to dry, and then kept bottled for use. This solution is delicate for 0.003 per cent. HCl. Combined HCl as well as acid salts and inorganic acids, in the concentration in which they occur in the stomach, will not

cause this solution or the prepared paper to become pink. A pink reaction denotes the presence of free hydrochloric acid.

2. Gunzberg's Phloroglucin Vanillin (for reagent see Appendix).—This reagent, if active, is of a pale-yellow color. It darkens and deteriorates on exposure to light, so should be kept in a dark-colored bottle or prepared fresh each time that it is required. A drop or two of this reagent is placed in a porcelain dish, together with an equal amount of filtered gastric contents, and heat gently applied until the liquid has evaporated. If HCl be present a rose-red color will appear at the margin of the evaporating fluid. This test is unmistakable and is most delicate, demonstrating the presence of free HCl in the proportion of 1 to 10,000 or 0.005 per cent. The reaction is not interfered with by albuminates, by salts present in the usual amount, or by organic acids.

QUANTITATIVE ESTIMATION OF TOTAL ACIDITY.

The reaction of the filtered gastric juice being determined by the congo-red or the tropeolin papers, the total acidity is next determined by titrating against a decinormal sodium hydrate solution.

Technic.—A Mohr's burette is filled to the "0" mark with standardized decinormal sodium hydrate solution.

To 10 cubic centimeters of filtered gastric juice in a porcelain dish, 10 cubic centimeters of distilled water is added, and one or two drops of a 1-per-cent. alcoholic solution of phenolphthalein added as an indicator. (This indicator is inactive in the presence of carbon dioxid.) In the presence of free acids this mixture is colorless. The sodium hydrate is now run in slowly with constant stirring, until the rose-color, which appears on the addition of each drop of alkaline solution, no longer disappears nor is intensified by further addition of the sodium hydroxid solution.

As a rule the acidity of the gastric contents, one hour after the ingestion of the test-breakfast, requires from 4 to 6 cubic centimeters of decinormal NaOH to neutralize 10 cubic centimeters of gastric contents. For example, suppose 5.2 cubic centimeters were required to neutralize 10 cubic centimeters of

gastric contents (this is within normal limits). The result of the estimation is usually expressed in parts of decinormal NaOH per 100 parts of gastric filtrate. This expression is easily obtained by moving the decimal point one place to the right. The result in the above example would therefore be recorded as 52 parts of decinormal NaOH per 100 parts of gastric contents.

QUANTITATIVE ESTIMATION OF FREE HYDROCHLORIC ACID.

To 10 cubic centimeters of filtered gastric juice add an equal quantity of distilled water, and one or two drops of Töpfer's reagent. This mixture placed in a porcelain dish is titrated with a decinormal solution of NaOH until the pink color of the solution has been entirely removed and is replaced by a pale-yellow. Suppose, for example, 6 cubic centimeters was the amount required to neutralize the free HCl contained in 10 cubic centimeters of gastric contents. To obtain the percentage of HCl in the gastric contents the following calculations are required:—

One centimeter of decinormal NaOH solution is equivalent to 0.00365 gram of HCl (decinormal NaOH is equivalent to 4 grams of NaOH dissolved in exactly 1000 cubic centimeters of distilled water; each 1 cubic centimeter of this solution should exactly neutralize 0.00365 gram HCl). Therefore 6 x 10 would equal the number of cubic centimeters of NaOH solution required for every 100 cubic centimeters of gastric contents, and the result, 60 x 0.00365, would equal the percentage of HCl in the specimen under examination, which would be 0.219 per cent. HCl.

Causes of Lowered Gastric Secretion.—I. In acute and chronic gastric inflammation.

II. Atrophy of the gastric mucosa from any cause.

III. General functional depression.

IV. Expression of a gastric neurosis.

V. Congenital peculiarity.

DETERMINATION OF ORGANIC ACIDS.

These include lactic and acetic, and the true fatty acids, particularly butyric. Acetic and fatty acids are not found dur-

ing normal digestion, and if present, as they sometimes are, have either been introduced with the food or have been produced by fermentation of carbohydrates set up in the stomach by bacteria introduced with the saliva.

The physiologic presence of lactic acid during the first stage of gastric digestion, may be due either to its formation within the stomach, or from its having been introduced with the food, as in baked bread.

Uffleman's Test for Lactic Acid.—The addition of a few drops of filtered gastric contents to Uffleman's reagent (see Appendix) in a test-tube will, in the presence of lactic acid, change the amethyst-blue to a canary-yellow. This test is positive in the presence of 1 part of lactic acid in 20,000.

Sources of Error.—Lactates cause the same reaction. This is, however, immaterial, since we desire to recognize lactic acid whether free or combined. The reaction also takes place with alcohol, sugar, and certain salts, particularly the phosphates, but rarely in their usual concentration after the test-breakfast.

The fatty acids, particularly butyric, strike a tawny-yellow color with a reddish tinge with Uffleman's reagent. The reaction is positive with 1 part in 2000. Fatty acids may also be detected by heating to boiling a few cubic centimeters of gastric filtrate in a test-tube, over the mouth of which a strip of moist neutral litmus paper is placed. On this the vaporized volatile acid will produce the usual change.

Acetic acid is easily recognized by its odor, but it may also be detected by neutralizing a watery residue after the removal of an ethereal extract, with sodium carbonate, and then adding neutral ferric chlorid solution. In the presence of acetic acid a striking blood-red color will be produced. A similar reaction occurs in the presence of formic acid, but this acid is never present in gastric juice.

Alcohol.—This is sometimes formed in the stomach during intense yeast fermentation. This may be detected by the *Lisben's iodoform test,* applied as follows: To a portion of the gastric filtrate add a small portion of liquor potassii, and then a few drops of a solution of iodine and potassium iodide in water. (See Appendix.) If alcohol be present a yellowish scum gradually occurs on the surface, which is readily recognized as iodo-

form by its odor. The same reaction occurs in the presence of acetone, but occurs more rapidly.

Propeptone and Peptone.—These are products of albumin digestion, and when present indicate the activity of this part of the gastric function test. A few drops of filtrate added to hot Fehling's solution produces a purplish color, if they are present.

Starch.—The addition of a drop of Lugol's solution to a piece of filter paper upon which some unfiltered gastric contents has been allowed to fall will, in the presence of starch, yield a blue reaction. This reaction is intensified by the addition of a drop of crude nitric acid.

MICROSCOPIC EXAMINATION.

Examination of the unfiltered gastric contents under low power will reveal the excentrically marked oval starch grains, the budding yeasty cells, food *débris*, and epithelial cells, etc. Under pathological conditions we may find a great excess of epithelial cells, shreds of mucous membrane or fragments of new growths, various bacilli and sarcinæ, pus-cells, blood-cells, and bacteria.

TESTS FOR OCCULT BLOOD.

Blood may be intermittently present in the gastric contents or gastric vomitus as a result of one of the following conditions:—

I. Ulcer of the stomach or intestines.

II. Benign pyloric stenosis.

III. Spasm of the pylorus.

Occult blood is usually permanently present in cases of malignant disease of the stomach or esophagus.

Boas Modification of the Weber Test.—To 15 cubic centimeters of gastric contents, add an equal amount of ether and thoroughly agitate. To this mixture add 3 to 5 cubic centimeters of strong glacial acetic acid, and again agitate. Now allow the mixture to settle and decant 10 or 15 cubic centimeters of the clear supernatant liquid, and to this add 4 or 5 cubic

centimeters of ozonized oil of turpentine. In the presence of blood a violet or blue color will appear, which is intensified by the addition of chloroform. (See also test on page 154.)

Acetic Acid Ether-Guaiac Test.—To 10 cubic centimeters of gastric contents add 10 cubic centimeters of ether and 5 cubic centimeters of strong acetic acid. Thoroughly shake, and add two or three grains of powdered gum guaiac, and again agitate; allow to settle, and then add a few drops of fresh solution of hydrogen dioxid. In the presence of blood a purple or blue ring will appear at the line of contact, or the solution gradually assume a grayish-blue color.

ESTIMATION OF PEPTIC ACTIVITY.

The digestive power of the filtered gastric contents from the recovered test-meal depends upon, first, the amount of pepsin contained, and, second, upon the amount of free acid present, particularly the free hydrochloric acid. Artificial digestion is the only means at our command by which we may determine the peptic activity of the gastric juice.

Method of Ewald.—Prepare from the albumin of eggs, which have been boiled just sufficiently to cause firm coagulation, small discs or squares by first cutting thin slices of the coagulated albumin, and from these slices the cubes or discs. These bits of albumin may be prepared in bulk and preserved in glycerin, which is carefully washed off before using.

The Test.—Place an equal quantity (5 to 8 cubic centimeters) of filtered gastric juice in each of four test-tubes, add to each tube one or two pieces of the prepared albumin; then,

To tube 1 add nothing.

To tube 2 add one drop of HCl.

To tube 3 add four grains of pepsin.

To tube 4 add the above quantities of HCl and pepsin.

These tubes are now placed in an incubator at 37° C., and from time to time examined to note the progress of liquefaction of the albumin. By comparison we can roughly determine whether digestion is progressing normally in the unaltered tube, and whether pepsin, hydrochloric acid, or both, are necessary to accomplish it.

The results obtained from this investigation can only be considered in the light of an approximate indication of the peptic activity of the material tested.

According to Nierenstein and Schiff[3] it is necessary to differentiate three factors in order to arrive at an adequate idea of the significance of these pepsin determinations. These are: the diluting secretion, the pepsin secretion, and the hydrochloric acid secretion, all three of which must be regarded as distinct expressions of the secretory activity of the glandular parenchyma.

Following a better knowledge of the activity of these different factors, two improved modifications of the method of pepsin determination have been advanced. These are the methods of Hammerschlag and of Mett.[4]

Method of Hammerschlag.—Briefly outlined the method depends upon the activity of a few centimeters of gastric juice upon a 1-per-cent. filtered solution of egg albumin. Two test-tubes, one filled with the albumin solution alone and the other with the albumin solution, plus the gastric filtrate, are incubated for an hour at 37° C. At the expiration of this time the albumin content of each tube is estimated volumetrically, according to the method of Esbach (see page 198). The difference between the albumin precipitate in the two tubes is equal to the amount of albumin digested, and therefore is a measure of the peptic activity of the gastric juice. The objections to this method are, that the Esbach method is not very accurate, and also that albumoses are partly precipitated by the reagent. However, this method is sufficiently accurate for the ordinary clinical purposes, and when the Mett method cannot be followed, may be adopted. It would be better, however, to employ a method which employs diluted gastric juice, as in the Mett method.

Mett's Method of Peptic Determination.—Glass capillary tubes from 1 to 2 millimeter[5] diameter and of convenient length, are filled by suction with the fluid portion of egg albumin. In order to avoid accidental variation in the egg albumin, it is better to use the albumin of several eggs mixed. These tubes as they are filled should have their ends plugged with

[3] Arch. f. Verdauungskrankh, Vol. iii, 1902.
[4] Sahli's Diagnosis.
[5] J. A. D., Petersburg, 1889, from Palow's Laboratory.

bread crumbs to prevent loss of contents before coagulation. After filling they are placed horizontally in a simmering water bath, where they are allowed to remain for from four to five minutes. While boiling, the tubes should be kept in motion to insure uniform coagulation of the albumin. When freshly made, the albumin tubes contain innumerable bubbles which, however, gradually disappear, after four or five days the tubes are ready for use. After boiling, the tubes are wiped dry and the ends closed with sealing wax or stick lac to prevent drying. Thus prepared a stock of tubes may be kept indefinitely, but it should be ascertained before using that the albumin is still evenly in contact with the walls of the tube; if they have dried out they are not fit for use.

These tubes are cut as needed into lengths of from 2 to 3 centimeters. This cutting is best accomplished by nicking the glass with a small triangular file, when a quick bend at this point will usually cause accurate fracture of both tube and albumin. Portions containing air bubbles or showing irregular fracture, should be discarded.

Technic.[6]—For quantitative determinations of the peptic activity of the filtered gastric contents, employ a dilution of 1 to 16, using as a diluent $\frac{n}{10}$ HCl, using for each test 1 cubic centimeter of contents and 15 cubic centimeters 20th normal HCl.

This mixture is placed in a covered Stender[7] dish after two Mett's tubes have been placed in it, and the specimen allowed to digest in an incubator (37° C.) for twenty-four hours. At the end of this period the digested cylinders of egg albumin at each of the four ends are measured off and the average reading calculated. The relative amount of pepsin is then obtained by squaring the result (Shurtz's law), and if desired multiplying by the dilution, *e.g.*, 16. For making the measurements a pair of callipers with a vernier reading 0.1 of a millimeter, is convenient. A lens is useful principally for reading the vernier. One end of the tube is placed against one jaw of the calliper, and the other is separated until its end is just visible through

[6] After Sailer and Farr, U. of Pa. Med. Bul., Oct. 1906.

[7] A Stender dish is similar to a Petri dish, but is deeper, with a flat ground glass lid.

the opalescent edge of albumin. If the tube is at all oblique the shortest side is taken, while if the albumin is at all uneven, the highest point to which digestion has extended is taken. In place of the callipers the ordinary mechanical stage, which is usually fitted with a vernier scale, may be used.

Nierenstein and Schiff have proven that a dilution of at least 1 to 16 is absolutely necessary if we wish to obtain a relative idea of the quantity of pepsin as estimated from the digestion length. This is because with a dilution of less than 16, the length of digestion decreases as the length of time increases, owing to the concentration and activity of the inhibiting substances. This great dilution also decreases the amount of pepsin in the mixture, so that the digestion length is kept within the limits of Shurtz's law (3.6 millimeters in twenty-four hours). As there are instances when this dilution is not sufficient (with very active pepsin solutions), it may become necessary when the digestion length exceeds 3.6 millimeters with the 16-fold dilution, to repeat the pepsin test with a dilution of 1 : 32 ; then by squaring the length as before, and multiplying this quantity by 32, the relative amount of pepsin in the undiluted gastric contents is obtained. It is clear that in this estimation the unit of the relative amount of pepsin will be that quantity of pepsin by which 1 millimeter of albumin in the Mett tube will be digested in twenty-four hours with an acidity of 0.18 HCl. In this determination we do not consider the absolute quantity of pepsin, but simply the degree of concentration, for the result of Mett's method is the same whether large or small quantities of digestive substances are employed.[8]

Summary.—Conclusions of Sailer and Farr regarding variations due to alterations in technic[9]:—

1. Difference in the caliber of the tubes. The variations in the reading of the two ends of one tube were as great as between tubes of different caliber for tubes between 1 and 2 millimeters. In tubes greater than 2 millimeters the rate of digestion seemed to be uniformly greater in tubes of larger diameter.

2. The age of the tubes. Provided there is no separation

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[8] Sahli's Diagnosis, fourth edition, 1905.

[9] Sailer and Farr, *loc. cit.*

of the albumin nor putrefactive softening, age does not seem to affect the tubes. The method of preparation tends to make them sterile. Tubes that are too fresh (less than four or five days old) are said to digest more rapidly than tubes that are "ripe."

3. Variability in the digestion of albumin from different eggs is an unimportant factor.

4. The degree of digestion within certain limits is said to vary directly with the duration of digestion.

5. Variations in the temperature of the incubator. This is undoubtedly an important factor, but there are no definite data to offer.

6. The effect of variations in the acidity of the original specimen. This has been provided against in the technic of the method, since the use of so large an amount of diluting acid solution tends to render the acidity of the diluted specimen uniform in every instance.

ESTIMATION OF THE ACTIVITY OF RENNEN OR MILK-CURDLING FERMENT

Normal gastric juice contains, besides hydrochloric acid and pepsin, the rennen ferment as a natural secretory product of the gastric mucosa. Rennen possesses the property of coagulating milk without the presence or assistance of acids.

Method of Leo.—To 10 cubic centimeters of fresh, uncooked neutral or amphoteric milk, add from two to five drops of filtered gastric juice, and place the mixture in an incubator at 37° C. If rennen is present in normal amount, curdling should take place in from ten to fifteen minutes. In this process the slight amount of acid contained in the gastric filtrate is insufficient to cause coagulation. If curdling takes place very slowly it is questionable whether this change is due to the action of the rennen or to the formation of lactic acid, so to be exact, the reaction of the mixture should be taken before and after curdling has occurred. The rennen reaction is certain to have occurred only in the presence of an unchanged reaction. If coagulation does not occur within an hour rennen can be considered absent. As a further guide it may be remembered that the characteristic curd from rennen is a cake of

casein floating on clear serum, while the curd from lactic acid is lumpy and broken.

DIGESTION OF STARCH AND SUGAR.

During digestion starch is converted into grape-sugar, while cane-sugar is converted into invert-sugar (a mixture of cane- and grape-sugar). This action is instituted by the ptyalin of the saliva, and continues in the stomach in the presence of low acidity. It is later arrested by the increasing acidity, and is completed in the intestine by the amylopsin of the pancreatic juice.

Starch is recognized by the deep blue color produced by the addition of a dilute solution of iodine or Lugol's solution. This reaction grows less vivid as the starch is converted. If starch digestion is unduly delayed or does not occur, we may infer hyperacidity of the gastric juice.

DETERMINATION OF THE RATE OF ABSORPTION FROM THE STOMACH.

Penzoldt's Method. — A capsule containing potassium iodide (one and one-half grain) is swallowed. The appearance of the iodine reaction in the saliva indicates that absorption has occurred from the stomach. To test for the iodide in the saliva, paper moistened with starch paste and dried is used. After the capsule has been swallowed, the paper is moistened with saliva at short regular intervals, and then touched with a glass rod dipped in commercial (better fuming) nitric acid. Upon the appearance of iodine in the saliva the characteristic blue reaction occurs.

When absorption is normal this reaction is usually positive in from ten to fifteen minutes; but if absorption is delayed, the reaction may be slow in appearing or occur not at all.

TEST OF THE MOTOR-FUNCTION OF THE STOMACH.

If attempted extraction of a full meal six hours after ingestion fails when properly performed, or if nothing can be

recovered from an Ewald test-breakfast after two and one-half hours, the motor function of the stomach may be considered normal.

A SECOND METHOD is to administer a large dose (ten or fifteen grains) of salol and test the urine at definite periods for the appearance of the products of its decomposition. The components of salol, carbolic and salicylic acid, are separated in the alkaline juice of the small intestine. They remain unchanged and undissolved during gastric digestion. Salicylic acid is readily detected in the urine by the violet color produced by the addition of neutral ferric chlorid solution. This test is conveniently performed by moistening filter-paper and bringing a drop of the ferric chlorid solution in contact with it. If gastric peristalsis is normal salicylic acid should begin to appear in the urine from forty to sixty or seventy-five minutes after ingestion of fifteen grains of salol.

IODOFORM METHOD.—Give with the test-breakfast one gram of iodoform in a well sealed capsule. The iodoform being insoluble in the gastric juice, will not be absorbed by the stomach, but will be passed on by peristaltic action to the intestine. By the action of the fluids of the duodenum the iodoform is decomposed with the formation of soluble sodium iodide. The demonstration of the iodide in the saliva by means of starch paper and nitrous acid, will indicate the time when the gastric contents is being discharged into the intestine. Iodine should normally be detected in the saliva in from one hour to one hour and a half after the ingestion of the capsule.

ROENTGEN RAY EXAMINATION.

With the advent of the Roentgen ray and its practical application, it has become an invaluable aid to diagnosis. A number of competent men have applied this agent in the study of the digestive tract, and have reached so many valuable conclusions pertaining to the size, location, motility, etc., of the stomach, that the examination of a patient suffering with any disorder of the digestive tract must be considered incomplete unless a Roentgenologic examination has been made.

The following quotation from an article by an expert in

this method of examination and treatment shows the present attitude of the profession upon this point[10]:—

"The superiority of this method of examination over others is recognized, I think, by all who have investigated sufficiently, and the subject has reached a stage of development when no case

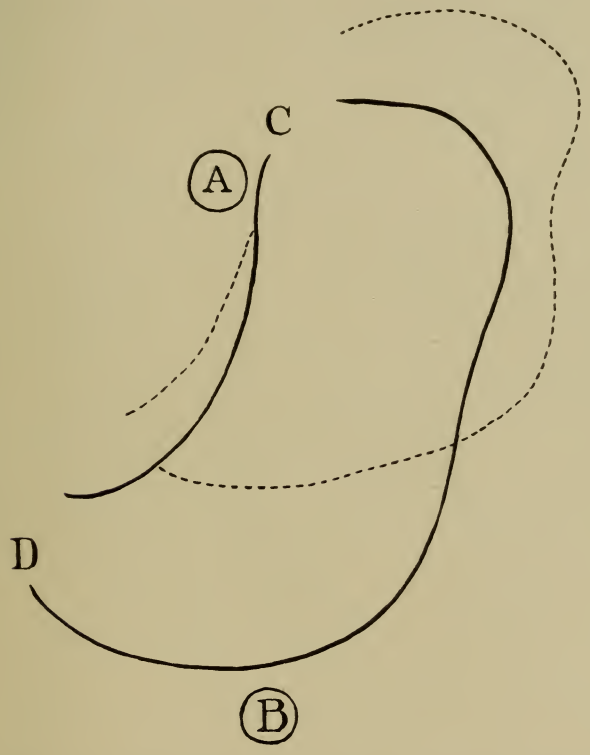


FIG. 17.—SOLID LINE SHOWS FORM AND RELATION OF TYPICAL NORMAL STOMACH, WITH SUBJECT IN STANDING POSTURE. DOTTED LINE SHOWS ALTERATION IN POSITION OF STOMACH, THE RESULT OF CONTRACTION OF THE ABDOMINAL MUSCLES.

A, Location of Ensiform. B, Umbilicus. C, Cardia. D, Pylorus.

involving chronic ailment of the alimentary canal is completely investigated until there has been a Roentgenologic examination by a competent man." From a careful examination of a number of normal individuals by the same observer, the following outline

[10] G. E. Pfahler, Jour. Amer. Med. Asso., Dec. 21, 1907.

may be considered to be that of a normal stomach in a healthy individual:—

When empty or moderately filled the stomach (Figs. 17, 18 and Plate II) occupies the left side of the abdomen and extends from the inner two-thirds of the left dome of the diaphragm to the median line just above the umbilicus. The upper two-

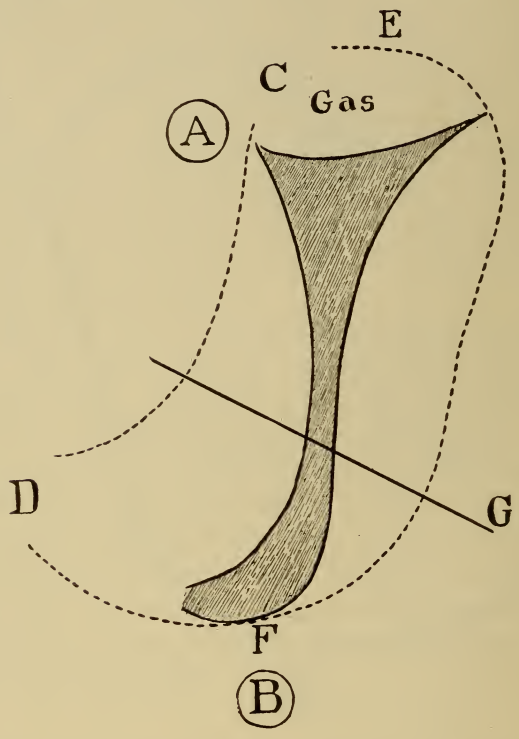
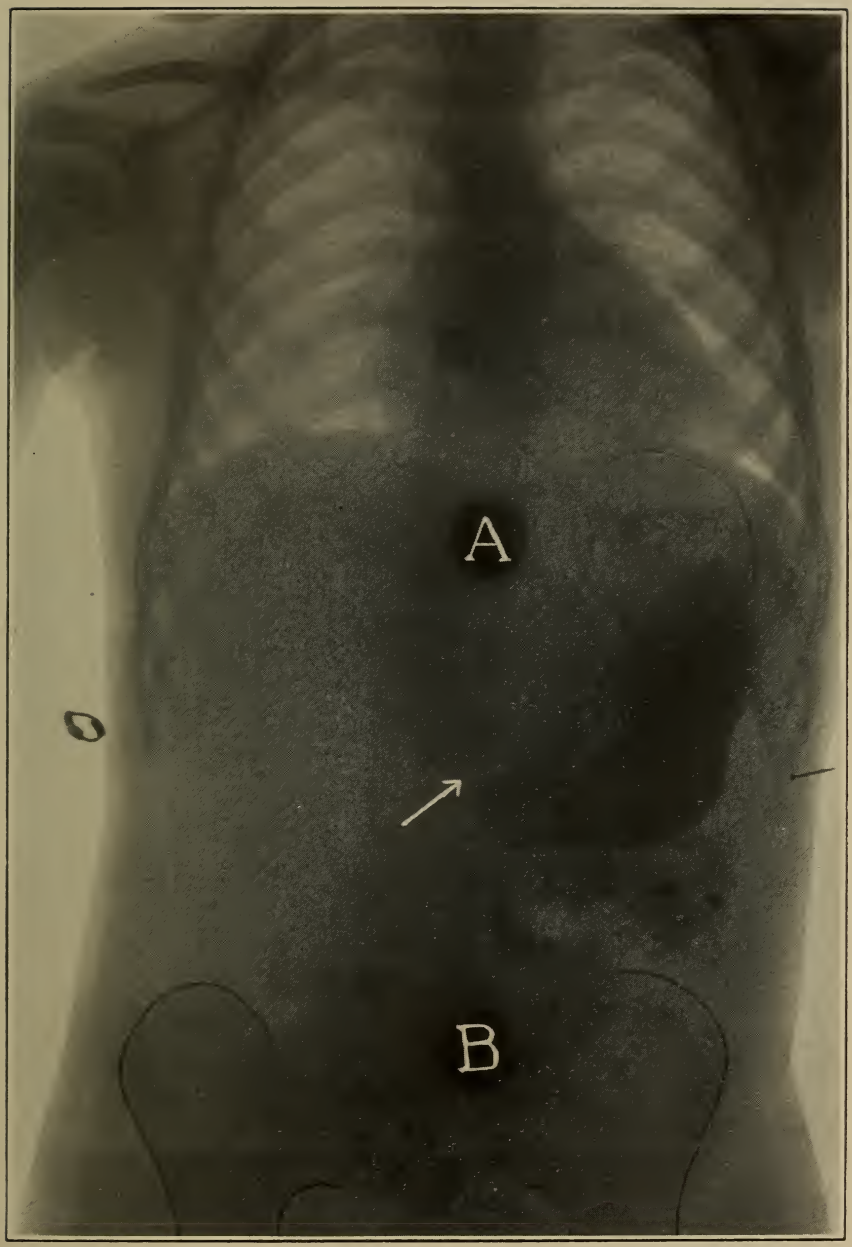


FIG. 18.—DOTTED LINE SHOWS FORM AND LOCATION OF NORMAL STOMACH. SHADED PORTION SHOWS CONTRACTION AND CHANGE IN FORM OF STOMACH DURING FILLING.

A, Ensiform. B, Umbilicus. C, Cardia. D, Pylorus. E, Upper Pole. F, Lower Pole. G, Showing place where Peristaltic Waves begin.

thirds is almost vertical, and the lower third is almost horizontal, making the general direction of the stomach slightly oblique. In the normal stomach the pylorus is on a level with the lower pole. No difference has been recognized between the normal type in children and in adults.

PLATE II.



NORMAL STOMACH. CHILD, AGE 4 YEARS.

Instantaneous Postero-anterior Exposure in Standing Posture. Shows Normal Stomach in Normal Position and its Relation to the Heart, Diaphragm, Spinal Column and Pelvis. A. Ensiform Cartilage. B. Umbilicus. Arrow Indicates Location of Pylorus. (*Radiograph by Dr. G. E. Pfahler.*)

The stomach of the average individual, in the standing position, extends to or below the umbilicus. It is vertical for more than its upper two-thirds. It occupies the left side of the abdomen, except when distended. The pyloric portion extends for one to two inches beyond the median line to the right.

The stomach is normally a very movable organ, as shown by the fact that about two-thirds of it crosses the median line when the patient lies upon the right side. When distended with food the pylorus is carried downward and to the right.

In summing up the results of his studies of the alimentary canal by the Roentgenologic method, Dr. Pfahler arrives at the following conclusions pertaining to its utility in diseases of the stomach:—

"1. The Roentgen examination will demonstrate obstructive disease anywhere along the alimentary canal, and much information concerning its character may be obtained.

"2. The Roentgen method is probably the most useful in the study of the stomach. By this means the size, form, position, motility, effects of massage, respiratory movements, abdominal contractions, peristaltic action, and the effect of food can be studied.

"3. The Roentgen rays are valuable in collecting additional data in the diagnosis of carcinoma of the stomach, but must not be depended upon to make an absolute diagnosis.

"4. The Roentgenoscopic and the Roentgenographic method of examination each has its advantage. Roentgenoscopically we study the motion of the viscera, while Roentgenographically we make accurate records and often obtain finer detail."

Technic of the Method.—*Preparation of the Patient:* The stomach and bowels should be empty, if possible. A purgative should be given the day before, and if the bowels are not moved an enema should precede the examination. Depending on the information desired, an ounce of bismuth may be given with the full meal, and this mixed with the food by massage or muscular contraction, or the bismuth-kefir mixture (bismuth subcarbonate, one ounce; kefir, one pint) may be given.

When carcinoma is suspected, no food should be given before the examination because the lodgment of food in the stomach may give a picture suggestive of neoplasm. Men need no

special instructions regarding dress. Women should wear a kimona or special muslin gown. This special muslin gown hangs from the shoulders and should be slit in front at the level of the pubes and posteriorly in the lumbar region for the distance of ten inches. These slits are closed by strings. Through them the necessary land marks are determined, and the cents are attached to the ensiform cartilage and the umbilicus by adhesive plaster.

Apparatus.—Besides the usual apparatus for Roentgenographic examination, appliances are necessary which will enable the operator to make the observations and negatives in any position. Roentgenoscopic apparatus is also necessary, and a dark room which is absolutely dark. Dr. Pfahler has recently adapted a dark room for this purpose. All the apparatus is in an adjoining room. The rays are passed through a door into the dark room before reaching the patient. Therefore all light is excluded and the patient is not frightened by the noise of the machine, nor is there danger of the patient touching the wires. In addition this door cuts off the soft rays and make it practically impossible for the skin of the patient to be burnt during the observations.

It is necessary in order to study the shadows on the screen that the operator should remain in the dark room for fifteen minutes before beginning the examination.

In order to study both the stomach and the colon at the same time, an ounce of bismuth mixture may be given and allowed to pass to the colon. After twenty-four hours another ounce of bismuth is given, when the outlines of the stomach and colon will appear in their relative positions.

X.

THE FECES.

PHYSICAL CHARACTERISTICS.

The Number.—One stool per day is the normal average for a healthy adult. Three daily, or one in forty-eight hours, may be normal for some individuals, and not incompatible with health.

The Reaction.—Whether the stools be acid or alkaline is of no special clinical importance. In adults the reaction is usually alkaline, sometimes neutral, but rarely acid. Acid stools are the rule in infants.

The Amount.—The amount varies in proportion to the amount of solids ingested. A preponderance of vegetable food usually produces a large quantity, while animal food leaves comparatively little residue.

The average daily amount of feces varies between 60 and 250 grams, of which 75 per cent. is water.

To weigh solid feces ascertain the weight of both the feces and their container, then weigh the container empty, and subtract the latter from the former weight, which will represent the weight of the contained feces. If the feces are liquid they may be measured, and the amount expressed in cubic centimeters.

The Odor.—The disagreeable odor is largely due to the presence of indol and skatol, but may be further increased by hydrogen sulphide, methane, and methyl-mercaptan.

The Consistence.—This varies greatly and depends largely upon the amount of fluids ingested, the temperature, the climate, and the condition of the digestive tract. In man the usual form is the characteristic plastic cylinder. Clinically expressed, the consistence of the feces may be liquid, mushy, or solid or formed.

Serous Stools.—These are composed of fluid without fecal matter, and are of considerable diagnostic importance. Such

stools are characteristic of Asiatic cholera, cholera morbus, cholera infantum, and poisoning by antimony. In cancer of the rectum evacuations are small, frequent, and serous. Arsenic poisoning and acute catarrhal enteritis may produce this form of stool. Poisoning by toad-stools is also a frequent cause of serous stools.

The Color.—The color varies with the character of the food ingested, and is usually little influenced by the decomposition products of the biliary pigments. In adults, the color usually varies from a light to a dark-brown. Lack of color in the stools may occur in health, owing to the over-oxidation of the coloring matter into colorless products of bilirubin. Such stools do not necessarily indicate the presence of large amounts of fat nor obstruction of the biliary passages.

In infants, fat and undigested milk produce a whitish-color tinged with bile-pigments.

GREEN STOOLS are seen after taking calomel. After exposure to the air such stools are usually acid.

YELLOW STOOLS may be caused by the ingestion of santonin, rhubarb, or senna. Typhoid stools are yellowish-brown and have received the name of "pea-soup stools."

WHITE or CLAY-COLORED STOOLS frequently denote the absence of bile, as in obstructive jaundice.

Very dark or BLACK STOOLS may result from the administration of iron, manganese or bismuth, or from the ingestion of much meat, blackberries or red-wine.

"TARRY" STOOLS usually denote hemorrhage.

EXAMINATION OF INTESTINAL DIGESTION BY MEANS OF GLUTOID CAPSULES.

Sahli recommends the use of glutoid capsules which are made from gelatin hardened in formaldehyde. These either do not dissolve in the gastric juice at all, or only after a considerable time, but are rather quickly soluble in the normal intestinal juices.[1] These are preferable to the keratin coated pills which were originally recommended, and are useful to diagnose the condition of intestinal digestion, *e.g.*, the pancreatic func-

[1] Deutch. Med. Woch., No. 1, 1897.

tion. They are filled with some substance which does not diffuse through the capsule wall, and whose absorption may be studied by an examination of the saliva or the urine. For diagnostic purposes glutoid capsules, containing two grains (.15 milligram) of iodoform and four grains (.26 gram) salol, are convenient.

In order to make the conditions of the test as uniform as possible in regard to the length of time the capsules remain in the stomach and in regard to the degree of digestive stimulation, it is advisable to administer the capsule with the test-breakfast. Experience has shown that normally, under the best conditions, *i.e.,* normal gastric motility, normal intestinal digestion, and normal intestinal absorption, iodine reaction may be expected to appear in the saliva, and salicyluric reaction in the urine in from four to six hours.

If one wishes to test the accuracy of any particular lot of capsules, it will be necessary to prove that this reaction time is obtained in healthy normal individuals. Only rough approximate differences in the time of the reaction are of clinical importance, so that it is sufficient to examine the saliva and the urine after six, ten, and twenty-four hours. The best results from this method are obtained by administering the capsule in the morning upon an empty stomach, and then, four hours later, allowing the patient to resume his meals as usual. The specimens of urine and saliva may, of course, be saved, and examined afterward.

DETECTION OF IODINE IN THE URINE OR SALIVA.

A few cubic centimeters of the fluid to be examined are boiled with a small piece of starch about the size of a pea, until the latter is dissolved. After cooling, the mixture is carefully overlaid upon nitric acid. If iodine is present a blue-violet ring, that gradually disappears, is formed at the line of junction of the two fluids.

A SECOND METHOD is to add to the urine a few drops of crude nitric acid and a less number of drops of chloroform, and agitate the mixture gently. If iodine be present the chloroform, which settles to the bottom, will be tinged rose-red or violet.

Both of the above tests are very delicate. But if the urine contains a very slight trace of iodine the chloroform-test is not very conclusive, since indol, skatol, and urorosein pigments may tinge the chloroform red.

DETECTION OF SALICYLURIC ACID IN THE URINE.

Dilute ferric chloride is added to the urine drop by drop. If the latter becomes a more or less intense violet the reaction is positive.

DETERMINATION OF THE MOTOR FUNCTIONS OF THE GASTRO-INTESTINAL TRACT.

Method of Adolph Schmidt.[2]—Schmidt demands two conditions for a satisfactory clinical method of examining the feces: 1. A knowledge of what a normal stool should be under a certain diet, involving the use of a "test-diet." 2. The methods of examination must be so simple that they are within the reach of every physician.

The Test-Diet.—The requirements are: *(a)* That it be nutritious enough to furnish calories sufficient for the body's needs. *(b)* That it be so constituted that it can be readily obtained in any household or hospital dietary. *(c)* That it contains a known amount of its constituents, so that variations in digestion and absorption can be detected in the stool.

Schmidt suggests the diet which is given below, followed by the approximate equivalents as sugested by Steele:—

Milk, 1.5 liters (2¾ pints).
Zwieback, 100 grams (3 ounces well-dried toast).
Two eggs.
Butter, 50 grams (1½ ounces).
Beef, very rare or raw, 125 grams (¼ pound).
Potatoes, 190 grams (6 ounces).
Gruel made from 60 grams oat-meal (2½ ounces).
Sugar, 20 grams (½ ounce).
This may be given somewhat as follows:—

[2] Quoted by Steele, Medical News, Dec., 1905.

Breakfast.—Two eggs, half of the amount of toast and butter, 2 glasses of milk, oatmeal, and sugar.

Dinner.—The steak and potatoes, ⅓ of the amount of toast and butter, 1½ glasses of milk.

Supper.—Two glasses of milk, remainder of toast and butter.

This diet is sufficient for the needs of the average patient, and while not offering much variety, is only taken for two or three days. A capsule containing five grains of charcoal is given with the first meal, and no examination made until the charcoal (black) appears in the stool.

The amount of each article composing the day's dietary must first be accurately determined and compared to the contents of convenient measures, so that the articles need not be weighed every meal. This does not apply to the meat and potatoes which should be weighed each day.

The Period of Passage.—The period of time required for food to pass through the intestinal tract can easily be determined by watching for the black in the stool. It is quite as necessary to know the time required for the passage of chyme through the gastro-intestinal tract, as it is to ascertain how often the stools occur, since the two are in no way identical.

It has been shown in chronic colitis with several watery stools a day, that the period of passage may be normal, and that peristalsis is decidedly increased only in the colon. According to Strass, using a diet similar to Schmidt, the time required for food to pass through the alimentary canal varies normally between ten and thirty hours. Under abnormal conditions this may vary between four and forty-eight hours.

The Specimen.—After the appearance of the charcoal, which indicates the beginning of the passage of the test-diet, a portion of one passage is collected in a clean receptacle and transferred to a clean, wide-mouthed bottle, and conveyed to the laboratory for examination.

Much can be determined from a naked-eye examination of such a mass. The technic for this examination, as outlined by Steele, is as follows:—

Take a piece of formed stool about the size of an English walnut or an approximate equivalent of liquid feces, and rub it

up in a mortar with distilled water until quite smooth and liquid. Part of this is then poured into a Petri dish and examined in a good light on a dark background. A little experience will enable the observer to acquire a large amount of information about the composition of the stool by this macroscopic examination.

The Gross Appearance of Normal Stools.—In normal digestion nothing should be seen by the naked eye, save a varying number of brown points (oatmeal husks), cellulose and indigestible parts of food, and occasionally sago-like grains that look like mucus, but which are found upon microscopic examination to be grains of potato. If, on the addition of the water, fat globules are seen, they must be considered pathologic unless the patient is known to have ingested excessive amounts of fats, as olive or castor oil.

Gross Appearance of Pathologic Stools.—(a) *Mucus* in large or small flakes is not affected by rubbing in the mortar. The mucus appears as glassy, translucent flakes often stained yellow by bile-pigment. Doubtful cases may be decided by the microscope.

(b) *Pus* in sufficient quantity to be recognized macroscopically, will have the characteristics of pus in any other region. It usually appears as small, yellowish streaks or collections throughout the stool.

(c) *Blood,* if in appreciable amount, will appear as dark reddish-brown or black; if from high in the digestive tube, becoming more nearly the color of fresh blood as the source of origin approaches the lower end of the bowel.

(d) *Parasites.*—Segments of tape-worm, also thread- and seat-worms, may be detected in varying numbers in patients so afflicted.

(e) *Foreign bodies* having entered the digestive tract, if indigestible and not impeded in their progress, may be recovered from the stool at varying periods after ingestion.

(f) *Calculi* from the various glands situated along the digestive tract, or from the intestinal canal itself (enteroliths), may be found in the feces, and appear as calcareous masses of various sizes and composition, depending upon their origin and age.

(g) *Remnants* of connective tissue and sinew from beefsteak. These can be detected by their whitish-yellow color and toughness, by which they can be distinguished from mucus. In case of doubt the piece should be examined microscopically with a drop of acetic acid. Connective tissue then loses its fibrous structure, while mucus becomes more thread-like. Small, single pieces of connective tissue can be found in normal stools.

(h) *Remnants of Muscle-Fiber.*—These appear as small reddish-brown threads or small irregular lumps.

(i) *Remnants of Potato.*—These appear like boiled grains of tapioca and may easily be confused with mucus. The microscope will show the true nature of these bodies.

(j) *Large crystals* of triple phosphate occur in foul stools, and can be recognized by their shape and by their solubility in all acids.

Microscopic Appearance of Normal Stools.[3]—Three slides are prepared from the liquid feces. The first is merely a drop of the material to be examined by both low and high power. The second slide is prepared by mixing a drop of acetic acid and a drop of the liquid feces upon a slide, heating it to boiling and then putting on a cover-glass. The third slide is prepared by mixing a drop of liquid feces with a drop of Lugol's solution.

Microscopic Examination of Slides. — *Slide* 1 will show *(a)* single small muscle fibers colored yellow with cross striation. Visible with a Leitz 3, but showing better with higher power. *(b)* Small and large yellow crystals of salts of the fatty acids. *(c)* Colorless (gray) particles of soap. *(d)* Single potato cells without distinguishable contents. *(e)* Particles of oatmeal and grain husks.

Slide 2.—A general idea of the fat-content of the stool may be obtained. Upon cooling, small drops of fatty acids may be found covering the whole preparation. The large crystals of salts of the fatty acids are broken up by the acetic acid, and fatty acids liberated. If the slide is heated again and examined hot, the fatty acids will be found to run together in drops which, as the slide cools, break suddenly apart.

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[3] Technic of E. Dutton Steele, Medical News, Dec. 16, 1905.

Slide 3.—Here should be found violet-blue grains in some of the potato cells, and small single blue points, probably fungi or spores.

Pathologic Microscopic Findings.—*Slide* 1. *(a)* Muscle fibers in excess, perhaps with yellow nuclei. *(b)* Neutral fat drops, or fatty acid, in soap crystals. *(c)* An excess of potato cells with more or less well-preserved contents. *(d)* Parasite eggs, mucus, connective tissue, pus, etc.

Slide 2.—Fatty-acid droplets in excess.

Slide 3.—Blue starch grains in the potato cells or free, oatmeal cells, fungi, spores or mycelia.

Formed Elements in the Feces.—(a) *Blood.* Red cells, if recently shed, may be distinguished by their characteristic form, or if disintegrated, only masses of brownish-red amorphous hematoidin will be found. In a certain percentage of cases characteristic crystals of hematoidin will be found.

(b) *Epithelial Cells.*—These are normally present in moderate numbers and represent the natural desquamation from the intestinal canal. They are more or less disintegrated, depending to some extent upon their height of origin in the intestinal canal, and upon the length of time they have remained free in the digestive tube. In catarrhal conditions they may be present in very large numbers, when they may assume diagnostic importance.

(c) *Pus Cells.*—These rapidly undergo decomposition, so that even when numerous and coming from a comparatively short distance above the rectum, they may be beyond recognition. The characteristic pus cell appears as a small round or slightly oval granular body. The presence of very much pus in the stools is indicative of rupture of an abscess into the intestinal tract.

CHEMICAL EXAMINATION.

This comprises only five routine tests: 1. The *reaction.* 2. The *sublimate* test for the condition of the bile salts. 3. The *fermentation* test. 4. The test for "lost albumin." 5. The test for occult blood.

1. The Reaction.—This is quite difficult to get with the ordinary litmus paper. It can be easily determined by dropping

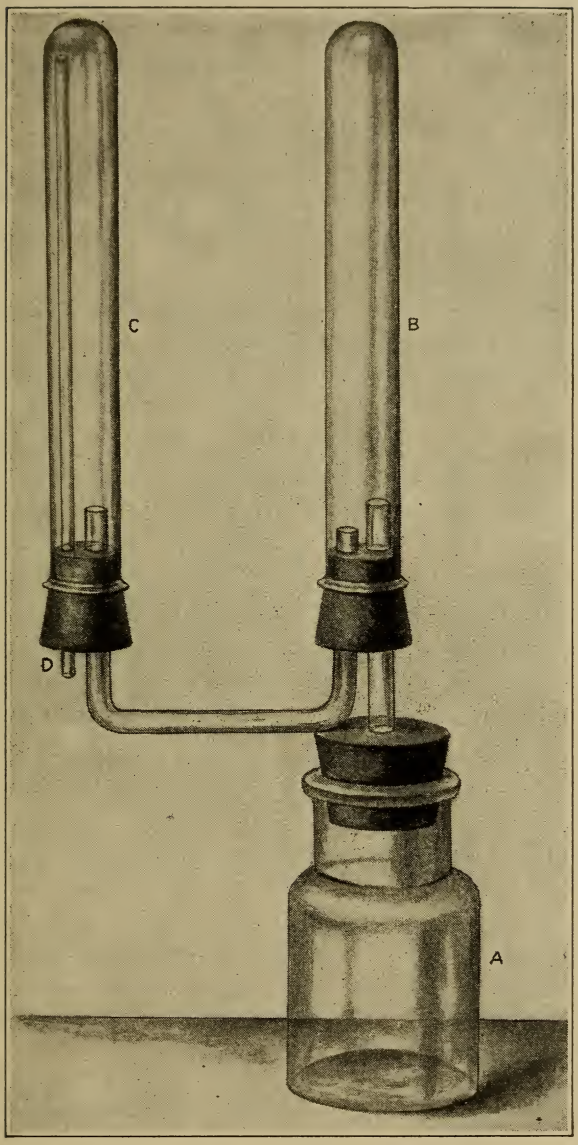

FIG. 19.—STRASBURGER APPARATUS, SHOWING ARRANGEMENT OF BOTTLES FOR FERMENTATION TEST OF FECES. (AFTER STEELE.)

a little softened fecal matter into 5 or 10 cubic centimeters of weak watery solution of neutral litmus, shaking it and noticing the color reaction.

2. The Sublimate Test.—This consists of taking a few cubic centimeters of liquid feces and mixing with an equal amount of a saturated watery solution of $HgCl_2$. A normal stool will quickly turn a pinkish-red, indicating the presence of hydrobilirubin, which will be more intense the fresher the material. A green color is pathologic and indicates the presence of unchanged bile-pigment.

3. Fermentation Test.—Described by Steele, using his modification of Strasburger's apparatus.

The apparatus consists of a two-ounce, wide-mouth bottle A (see Fig. 19). This is fitted with a perforated cork through which runs a tube to the test-tube B, which is also fitted with a rubber cork with two perforations. A bent tube runs from the tube B to the test-tube C, to allow for the escape of air. Each tube has a capacity of a little more than 30 cubic centimeters when fitted on the corks. The apparatus is simple and easily constructed, and broken parts can be replaced readily.

The Test.—About five grams of solid feces, or an equivalent of liquid feces, are rubbed up with a little distilled water and placed in the main bottle A. This is filled with sterile water, the tube is filled with water and fitted into place (not necessarily full), and tube C is then fitted on empty. The reaction is carefully noted before the test is started. The apparatus is then stood in a warm place—best in an incubator at 37° C. for twenty-four hours. If gas forms by fermentation in A it will rise into B, and the amount will be indicated by the amount of water displaced into C. Normally the fermentation test should show practically no gas, and the original reaction of the material should be unaltered in twenty-four hours. If more than one-third of the tube C is filled, it is pathologic. If the reaction after twenty-four hours is decidedly more acid, it is a carbohydrate fermentation. If alkaline, and with a foul smell, it is a fermentation of the albumins.

4. Test for Lost Albumin.—A portion of softened stool is filtered (a slow and difficult process). The filtrate is shaken with silicon and refiltered, then is saturated with acetic acid to

bring down the nucleo-proteids, and finally a drop of potassium ferrocyanide solution is added. A decided precipitate indicates albumin. A positive test shows only that there is a decided diminution in albumin digestion.

BLOOD IN THE STOOL.

If bleeding has its origin in the upper part of the digestive tract (stomach or small intestine), it is so altered by the action of the digestive fluid that, by the time it finally appears in the stool, it has a black or brownish-black appearance which has been likened to tar or coffee grounds. However, if the hemorrhage be large and the peristalsis very active, blood may appear almost unchanged in the stool, even when of high origin in the digestive tube.

Relatively small amounts of blood may be so changed and mixed with the feces that they cannot be detected by the naked eye or by the microscope. This is termed "occult" blood, and is only recognizable by chemical means.

Preliminary Technic.—Owing to the possibility of the presence of a positive reaction, resulting from the ingestion of hemoglobin-containing food in a normal individual, it becomes necessary to restrict the diet in order to eliminate this possible source of error, and to limit the test-diet by the administration of a capsule containing five or ten grains of charcoal, and to watch the stools for the appearance of the black discoloration due to the passage of the charcoal. Only after the appearance of the charcoal in the feces should a specimen be taken for the test for occult blood.

Steele[4] recommends a "liquid diet," including milk and broths, or a "semi-liquid diet" composed of milk, eggs, and toast, to which may be added moderate amounts of the ordinary winter vegetables.

Red meats and beef juice should positively be withheld. Steele has shown that iron, either in the organic or inorganic form, will even in large doses not affect the reaction.

As a preliminary to the test, it is necessary to eliminate as fully as possible extraneous sources of blood. Thus tuber-

[4] Amer. Jour. Med. Sci., July, 1905

culous ulcer, typhoid fever, hemorrhoids, fissure, and purpura should be excluded. Also ingestion of carmine, swallowed blood from any cause, hemoptysis, epistaxis, and menstruation.

Technic of Test.[5]—If the feces are solid they must be softened with distilled water. To 5 or 6 cubic centimeters of liquid feces, in a wide-mouthed cork-stoppered bottle, add about thrice as much ether and agitate or thoroughly mix by shaking. Then add a few grains of powdered guaiac and again agitate. Follow this by 5 cubic centimeters of glacial acetic acid (99.4 per cent.), and still again agitate. Allow this mixture to stand until the solid particles settle to the bottom, and then decant into each of two test-tubes 5 cubic centimeters of the supernatant liquid.

One test-tube should be kept as a control. To the other add 1 or 2 cubic centimeters of a fresh solution of hydrogen dioxid. If a bluish discoloration occurs either at the line of contact of the two solutions or throughout the mixture, the reaction is positive.

Significance of the Occult Blood.—According to Boas occult blood is constantly present in carcinoma of the gastro-intestinal tract; intermittently present in gastric and duodenal ulcers; occasionally in stenosis of the pylorus, and is absent in gastritis, hyperchlorhydria, and in the gastric neuroses.

BACTERIA AND PROTOZOA IN THE FECES.

There are many varieties of bacteria in the feces. They have been estimated to amount to about one-third of dried feces. Some are harmless at all times; others which are, under ordinary conditions, harmless may develop pathogenicity under certain circumstances.

Bacillus Coli Communis.—This organism is constantly present in the feces, and is normally non-pathogenic. It has, however, been found in pure culture in cases of appendicitis, empyema of the gall-bladder, pyelitis, and cystitis.

Morphology.[6]—It is a rod with rounded ends, sometimes so short as to appear almost spherical, while again it is seen with

[5] From Professor Daland's Laboratory.
[6] Abbott's Bacteriology.

very much longer threads. It may occur as single cells or joined together in pairs, end to end. It is motile, and does not form spores. It stains with the ordinary alkaline dyes, and is decolorized by Gram's method.

Compared to the typhoid bacillus, it will be found to be less motile; grows more rapidly on gelatine colonies, and luxuriantly on potato (typhoid growth usually invisible). Colon bacillus coagulates milk in incubator from thirty-six to seventy-two hours; typhoid does not. Colon bacillus decomposes sugar solutions; typhoid does not.[7]

Bacillus Typhosis.—This is a distinctly pathologic bacterium, and is the specific cause of typhoid fever. Its appearance in the stools is scanty, but usually can be isolated by appropriate methods during the first few days of the disease.

Pratt, Peabody, and Long[8] obtained it from the stools of only 31 per cent. of febrile cases, and none in twenty-one convalescents examined by them. They state that it occurs most in the blood, and that it does not develop in the intestinal contents except under unusual conditions. The bacillus in the intestines comes chiefly from the gall-bladder; frequently it is found in the urine in larger numbers than in the feces, and is found in greatest numbers in feces containing blood.

Morphology.—The bacillus is about three times as long as it is broad, with rounded ends. Its length may vary greatly, but its width remains fairly constant. It is actively motile, and Loeffler's method of staining will show it is possessed of many delicate flagelli (see methods of staining).

Its growth on potato usually is invisible. It does not cause coagulation of milk (colon bacillus does). Owing to the tendency to retraction of the protoplasm from the cell-envelope, and the consequent production of vacuoles in the bacilli, the staining is often irregular. It stains with the ordinary aniline dyes.

Bacillus lactis aërogenes and the bacillus **proteus vulgaris**, are probably always present in the stools. They are probably pathologic in many cases of cholera infantum.

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[7] For methods of plating, isolating, and culture, see works on bacteriology.
[8] Jour. Amer. Med. Asso., Sept. 7, 1907.

Streptococcus aërogenes.—This is an etiologic factor in certain cases of entero-colitis.

Bacillus Dysentericus (Shiga bacillus) is the cause of one form of infantile dysentery, and is generally present in the discharges of such patients showing blood and mucus.

The Comma Bacillus.—This is the infective agent in Asiatic or true cholera.

Morphology.—It is a slightly curved rod of a length of from one-half to two-thirds that of the tubercle bacillus, but thicker. Its curve is never very marked, it may be nearly straight. It is a flagellated organism, but has only one flagellum attached to one end. It is actively motile. It does not form spores.

Cultural Characteristics.—On gelatine plates at room-temperature, its development can be observed after as short a period as twelve hours. It rapidly liquefies gelatine. It is strictly aërobic. It takes the ordinary stains.

Diagnostic Method.—A smear is made from one of the small, slimy particles found in the semi-fluid evacuations, dried, fixed, and stained in the ordinary way. If upon microscopic examination only curved rods, or curved rods greatly in excess of all other organisms, are found, the diagnosis of Asiatic cholera most probably is correct.

Tubercle Bacillus.—This organism probably is always present in all cases of intestinal tuberculosis.

Diagnostic Technic (R. C. Rosenberger's).—A selection of "any part of feces is made, there being no effort at a selection of any particular mass or portion. If the stool is solid a small mass is mixed with sterile water. After drying and fixing, the spread is stained with carbol fuchsin for fifteen or twenty minutes, cold. The excess of stain is drained off and Pappenheim's solution (see Appendix) is applied, and when the preparation is the color of the counter stain (methylene-blue) thorough washing in distilled water is resorted to, the spread being then dried and mounted in balsam. The most important point in the technic is to obtain a spread the color of the counter stain with not a particle of the carbol-fuchsin showing to the naked eye."[9]

[9] Solis-Cohen: New York Med. Jour., Aug. 21, 1907.

It is alleged, but not conclusively determined, that no other acid- or alcohol-fast bacilli will withstand this method excepting the tubercle bacillus.

Ameba Dysenteriæ.—This is now generally conceded to be the specific cause of the so-called amebic dysentery. In typical cases the stools contain much blood-stained mucus, containing large numbers of these amebæ. These parasites so resemble epithelial cells that a positive diagnosis can only be reached when they are seen under the microscope to move and to extend their pseudopods. To keep them alive sufficiently long for examination, the feces must be caught in a warm pan and a portion immediately transferred to a warm microscope slide for examination. The organism is from 8 to 50 microns in diameter (see Plate I). To develop the nucleus the organisms should be obtained fresh; they are killed by the addition of a few drops of acetic acid or corrosive sublimate (sat. watery solution) while on the microscope stage. The nucleus is spherical and about 5 microns in diameter (see page 103).

For intestinal worms, ova, etc., see section on "Animal Parasites."

THE CLINICAL SIGNIFICANCE OF THE EXAMINATIONS.

Mucus.—As a rule, the appearance of mucus in the stool indicates the presence of inflammation of the mucous membrane, and is the one trustworthy sign of that condition (Steele). There are two conditions in which mucus has no significance: 1. When thin mucus spreads over the surface of a hard stool. 2. The so-called mucus-colitis with the discharge of mucus casts.

Bile-Pigment.—A green color of part or all the stool (by the sublimate test) is pathologic, except in children. It means too short a period of passage through the intestines. A fresh normal stool should give a pink reaction with $HgCl_2$. If a color reaction of any kind is absent it indicates either absence of bile from the intestine, a very fatty stool, or the reduction of hydrobilirubin (urobilin) into leucohydrobilirubin, a colorless product (Steele).

Fat.—It will require some practice in the use of the diet to tell whether there is an excess of fat in the stool or not. As the

normal amount of fat varies within wide limits in normal feces, only a great excess can be considered abnormal.

Remnants of Meat.—Normally there should be only microscopic particles of connective tissue and muscle fiber. An excess of either is often visible to the naked eye.

Excess of connective tissue means insufficient gastric digestion, because fibrous tissue is digested in the juices of the stomach only. Excess of undigested muscle-fiber means disturbance in intestinal digestion, and probably means trouble in the upper part of the small intestine, as follows: *(a)* Indicating a total lesion of the pancreas and absence of proteolytic and tryptic digestion. *(b)* Trypsin may be present, but its activating enzyme, enterokinase, may be absent. *(c)* The period of passage of the food through the intestine may be so rapid that no time is given for its digestion. *(d)* Large pieces of undigested muscle may be present, because gastric digestion is imperfect and the connective-tissue framework of the meat has not been properly removed.

Pathologic carbohydrate fermentation means poor starch digestion and indicates, as a rule, disturbance in the small intestine, which is usually due to insufficiency in the succus entericus.

Pathologic albumin fermentation means a large remainder of albumin in the feces, and indicates, in Schmidt's experience, serious trouble, usually some anatomic change in the mucous membrane of the small intestine (Steele).

FOREIGN BODIES, CALCULI, AND CONCRETIONS.

An attack of biliary colic or gall-stones may be followed by the appearance, from time to time, of gall-stones in the feces, or they may appear without the previous occurrence of any symptoms referable to the liver or its appendages. In searching for them, the feces should be rendered thoroughly fluid by rubbing in water and then by passage through a sieve. Various forms of simple and complicated apparatus are upon the market, and are known as *stool-sieves*. A very satisfactory home-made substitute is made by filling in a wire-frame with two or three layers of gauze or cheese-cloth, the wire-frame being arranged to fit around the rim of the bowl of the closet. To be certain of

not missing the stones it is necessary to examine all movements for at least fourteen days following the cessation of the attack of colic. Even a careful and prolonged search may fail to find the concretion in certain undoubted cases of gall-stone disease. Sometimes because the stone causing the symptoms was not passed, but after being jammed in the neck of the bladder finally returned into the cavity of the gall-bladder itself. Again, the stone may be retained for a long time in a fold or diverticulum of the intestinal tract, and finally the concretion may have fallen to pieces in the intestine. Another explanation of why the stones fail to appear is that typical attacks of colic may be due to inflammation without the presence of gall-stones.

Gall-stones are concretions which form in the biliary passages. They vary in size from a pin-head to that of a pigeon's egg or even larger. They are composed chiefly of cholesterin and calcium-bilirubin in varying proportions; besides these there are present minute amounts of other bile-oxidation products and calcium-carbonate. A predominance of cholesterin produces a light-, and of calcium-bilirubin a dark-colored stone, the absolute color varying usually between a light- or dark-brown to a dark-olive green. Stones vary greatly in hardness, and on cross-section usually show distinct concentric layers of crystalline substance, sometimes of different colors. The surfaces may present a beautiful faceted formation from attrition between a number of stones lying in the bladder, or they may be irregularly granular.

It is important not to confound other intestinal concretions with gall-tones. Woody bits of food, particularly the cores of pears, have been termed pseudogall-stones. The microscopic examination of bits of these scraped off with a knife, present the picture of characteristic wood cells. Chemical examination will also prevent mistake (see below).

So-called biliary sand, in most instances, consists of these small pseudogall-stones. The existence of true biliary sand has not yet been conclusively proven. Another kind of pseudogall-stone consists of balls of fat and fatty soaps which are not easily melted. They are found in the stools after the administration of large amounts of olive oil, as in a favorite method of treating cholelithiasis.

For *chemical examination* gall-stones are at first dried, then powdered and treated with alcoholic ether to extract the cholesterin. This may then easily be recognized by allowing the extract to evaporate upon a watch-glass, when the characteristic glistening rhomboids of cholesterin crystallize out, and may be easily recognized by the microscope. After extraction with alcohol and ether the residue is treated, while cold, with very dilute potassium hydroxid. If the powder contains calcium-bilirubin, a yellow solution will be obtained which gives Gmellin's reaction.

The much rarer PANCREATIC CONCRETIONS differ from gall-stones in that they contain no bile-coloring matter, and are composed chiefly of calcium-carbonate, which dissolves readily with effervescence in hydrochloric acid.

Intestinal Stones or Fecal Concretions.—These are supposed to play an important part in exciting attacks of appendicitis, but seldom appear in the stools. They consist almost exclusively of ammonio-magnesium phosphate (triple phosphate), and should be examined after the manner of urinary calculi (see page 226).[10]

[10] Sahli's Diagnosis.

XI.

THE URINE.

PART I.

GENERAL CONSIDERATIONS.

The normal constituents of the urine are usually tested for by quantitative methods, since we are concerned with the actual amount of these substances and not with their presence or absence from a given sample. In testing for abnormal constituents, on the other hand, we are concerned, as a rule, with their presence or absence, though in certain instances we may desire to know the absolute quantity of these abnormal substances.

The Sample.—If we are to make a qualitative examination for abnormal constituents in a single sample of urine, it is best to collect a specimen about three hours after the ingestion of a hearty meal (dinner), as such a sample is most likely to contain the substances sought (usually albumin or sugar).

Cases which at times show a trace of albumin will usually show an increase late in the day or after active exercise, and the collection of specimens should be timed to meet these conditions.

Method of Collection of Specimens.—The following is a copy of the directions furnished by Prof. Judson Daland for the instruction of his patients:—

Directions for Collecting Urine.—The urine should be collected in a perfectly clean vessel and four ounces sent to the laboratory. Wide-mouthed four-ounce bottles especially adapted for this purpose should be obtained at the drug stores, and when possible the urine should be directly passed into the bottle.

1. The evening specimen is to be obtained in the following manner. Empty the bladder immediately before the evening meal and discard this urine. From the urine first passed after the evening meal, take four ounces and note the hour when voided.

2. The second specimen is obtained from the urine first passed upon arising in the morning. Note the hour when the urine was passed.

To obtain the total quantity of urine passed in 24 hours. On the day when the observation is begun, at a definite hour, empty the bladder,

and discard this urine. All the urine passed afterwards is to be collected in a suitable, clean, dust-proof receptacle and kept in a cool place. The following day at the same hour, when the bladder was first emptied and the urine discarded, again empty the bladder. This urine should be added to complete the total amount for 24 hours, which should be expressed in ounces. After the total amount of urine has been collected and thoroughly mixed, send four ounces of the mixture.

Example. Observation began on January 1st., at 8 A.M. The bladder is emptied at 8 A.M., this urine is discarded; the urine passed during the day and night are saved. The next morning, January 2nd., at 8 A.M., the bladder is again emptied, and this urine is added to complete the total quantity for 24 hours. A label on which is written the name, date, and time when the urine is passed should be *pasted on the bottle.*

The Twenty-four Hour Specimen.—For accurate results, particularly by quantitative methods, it is necessary to examine a portion of the mixed urine voided during twenty-four hours. Such a specimen should be examined within six or twelve hours after the collection is complete; this will usually prevent errors due to the processes of decomposition and putrefaction which might destroy the formed elements.

Catheterized Specimen.—Catheterization is often resorted to in order to obtain urine free from contamination, which might enter it from the lower part of the urinary tract. For specimens of urine from one kidney the Harris segregator may be used, or in the cases of women, the ureters may be catheterized.

Urine kept at room-temperature, particularly in summer time, readily undergoes decomposition and putrefaction. These changes render it unfit for examination.

DECOMPOSITION CHANGES IN NORMAL URINE.

If no method of preservation of the sample is employed, the fresh urine, which is clear of acid reaction and showing no deposit, will gradually undergo the following changes:—

1. The lower part grows cloudy from sedimentation of mucus, cells and other detritis, the urine is still acid.

2. This sediment gradually settles to the bottom and may show minute crystals of uric acid, the urine is less acid.

3. Uniformly cloudy from beginning, precipitation of phosphates. The urine is very faintly acid or neutral.

4. Very turbid from precipitation of phosphates and development of bacteria. Copious sediment of triple and amorphous phosphates, bacteria, ammonium urate, and epithelial debris. Alkaline reaction and ammoniacal odor.

PRESERVATION OF SAMPLE.

When, for any reason, it becomes necessary to delay the examination of urine past the time when decomposition changes usually occur, these changes may be retarded in a number of ways: 1. By refrigeration. 2. By the addition of two or three grains of chloral for each ounce of urine. 3. By the addition of ten drops of 4-per-cent. formaldehyde solution for each ounce of urine. 4. By shaking with chloroform in the amount of five drops to the ounce of urine.

DESCRIPTION AND IMPORTANCE OF THE URINE.

The urine is an aqueous solution of the by-products of metabolism, so far as these are not excreted or eliminated by the lungs, bowels, or skin. It is the most important excretory product of the body, and is the medium through which the end-products of nitrogenous metabolism and the soluble mineral salts are almost exclusively excreted under normal conditions. Abnormal products of metabolism and many substances which have found their way into the circulation from without and which are foreign to the body, are likewise carried out in solution.

Not less than 50 per cent. of the total fluid ingested daily is excreted as urine.

General Characteristics.—Normal urine is perfectly transparent when voided, but soon becomes turbid, and on standing deposits a light flocculent sediment composed of a mucinous body and a few epithelial cells and leucocytes. If the urine is kept cold and care is exercised to exclude the entrance of micro-organisms, the upper portion of the urine will remain clear indefinitely. Ammoniacal fermentation, due to the activity of the *bacterium urea* and the *micrococcus urea*, causes cloudy urine from the precipitation of the phosphates.

Bacteria-free urine may, in winter, become cloudy because the contained urates are less soluble in cold urine than in warm urine. On standing the urates settle to the bottom, and the supernatant liquid remains clear as long as bacterial contamination does not occur.

Passage of Turbid Urine.—In man the passage of turbid urine is always abnormal, except during the first days of life,

when the turbidity is due to the profuse desquamation of epithelial cells and the relatively large amount of urates.

PHYSICAL CHARACTERISTICS OF THE URINE.

The Color.—The color of urine normally varies from a light yellow to a dark amber. This is largely influenced by the concentration of the secretion and by the reaction. The pigmentation is due chiefly to the presence of a substance called urochrome (derived from the biliary pigments) and indoxyl potassium sulphate (indican). Acid urine is always darker than alkaline; the color is naturally lighter when the excretion is abundant than when it is scant.

Deviations from the normal color are notably observed in certain diseases and during the administration of certain drugs. It may also occur in apparently healthy individuals in consequence of certain undetermined anomalies of metabolism.

Pale urine may occur as a neurosis or during the course of certain nervous diseases, particularly in epilepsy and hysteria. It may be a symptom of chronic nephritis and diabetes.

Dark urine, which is clear, occurs in the course of most acute fevers, and is due to the presence of uro-erythrin. A smoky color denotes the presence of decomposed blood, as in acute nephritis. *Blood-red* or *pink urine* usually denotes the presence of fresh blood. Recently Bar and Duaney[1] have called attention to a false bloody urine due to the activities of a pseudomembranous and chromogenic bacterium.

Yellow-brown or *greenish urine* suggests the presence of bile. *Brownish urine* occurs in melanosis and after the ingestion of rhubarb, senna, or tannic acid.

Smoky-brown urine indicates the presence of the end-products of ingested carbolic acid or its analogues.

Pale greenish urine, with a high specific gravity, usually indicates glucose.

White urine denotes the presence of pus or chyle.

Whitish turbidity denotes: 1. Pus. 2. Phosphates. 3. Ammonium urate.

[1] Le Progrès Medicale, Mar. 24, 1906.

URINE WHICH DARKENS ON STANDING.—Some urine, after standing for a variable period, becomes dark-brown or black. This may be due to the presence in the specimen of melanin or of phenol. Non-pathologic phenol urine or "carbol urine" may arise from medicinal or surgical treatment with phenol or closely allied compounds. Under these conditions the end-products in the urine usually appear as resorcin. Such urine, if alkaline when voided, rapidly darkens on exposure to the air, and if acid becomes dark more slowly, as the alkaline reaction gradually develops.

Under certain pathologic conditions the urine may darken on exposure to the air, owing to the presence of *alkapton*. This condition is known as *alkaptonuria*. A similar darkening in pathologic urine may result from the presence of a coloring matter termed *melanogen*.

Test.—To differentiate between the phenols and melanogen: Add bromine water to the suspected urine. In the presence of phenols there will be produced a permanent, yellow precipitate. A primary yellow precipitate which gradually darkens, becoming finally brown or black, indicates melanogen.

Corroborative Test.—Add to the fresh undarkened urine a few drops of dilute solution of neutral ferric chloride. A violet discoloration denotes phenols, brown or black denotes melanogen.

It must be remembered that urine containing alkapton will give many of the chemical tests for sugar which depend on a reduction process.

The Odor.—Freshly passed normal urine has a peculiar characteristic AROMATIC ODOR, resulting from the contained volatile acids. Decomposing urine has the characteristic odor due in part to the free ammonia resulting from the decomposition of urea.

Urine which is AMMONIACAL when freshly passed, points to a pathologic fermentation occurring in the bladder, usually accompanying cystitis.

A PUTRID odor denotes putrefactive change occurring in pus or other albuminous substances.

An odor resembling ACETONE is occasionally observed and denotes diabetes mellitus.

Urine containing cystin may, upon standing, develop the odor of HYDROGEN SULPHIDE.

Some articles of food, as asparagus and onions, and certain aromatic medicines, as turpentine and copaiba, may give characteristic odors.

THE AMOUNT.

The *average daily excretion* of urine in the United States is somewhat less than in foreign beer-drinking countries. A fairly normal average for the United States may be stated to be between 1200 and 1600 cubic centimeters, or about fifty ounces for men, while for women it is slightly less. Generally speaking, the amount will vary in inverse ratio to the insensible perspiration: hot weather diminishes and cold weather increases the amount. Profuse perspiration, sweating, vomiting, and diarrhea all decidedly diminish the amount. Children, and especially nursing infants, on account of the preponderance of liquid in their food, excrete a proportionately larger amount of urine, compared with body-weight, than do adults.

The normal amount of urine passed in twenty-four hours is consequently subject to wide variations, depending on the amount of fluid ingested, the character and the quantity of the food, the process of digestion, the blood-pressure, the surrounding temperature, the emotions, sleep, exercise, age, sex, and body-weight. During repose much less urine is excreted than during activity, hence the excretion during the night is less than during the day. The maximum secretion is usually observed during the first few hours succeeding a hearty meal.

Artificially the excretion may be increased by substances which have a tendency to raise blood-pressure, as tea, coffee, and alcohol. Many drugs bring about the same result. The most important of the medicinal diuretics are digitalis, squill, broom, juniper, nitrous ether, urea, etc. Distilled water also possesses distinct diuretic properties.

The quantity of urine in pathologic conditions depends, first on the condition of the secreting renal parenchyma and, secondly, upon the condition of the blood-current in the kidneys. It will therefore be affected in general by circulatory disturbances, as well as by disease of the kidney itself. To appre-

ciably alter the amount of the excretion, both kidneys must be diseased, for if one kidney be healthy it will assume vicariously the function of the other organ. This compensatory action regularly occurs after extirpation of one kidney.

As a rule the more *acute* the *nephritis* the more the excretion of urine will fall below normal, while the more chronic the process the more will the amount exceed normal. This increase reaches its maximum in the true contracted kidney where the volume excreted in twenty-four hours may be very great. *Diseases of the heart and lungs*, leading to chronic passive congestion, will diminish the total amount of the urine, a condition evidently dependent upon interference in the renal circulation.

It is evident, therefore, that the determination of the total output of the kidneys and an observation of the variation in the amount during the course of disease, may be of great diagnostic and prognostic value.

The increased excretion of urine following convulsions, particularly the hysterical variety and after attacks of angina pectoris, is probably dependent upon some vasomotor disturbance. Many alterations in the volume of the urine depend upon quantitative and qualitative variations in the substances eliminated, and are primarily induced by disturbances in metabolism.

Anuria, or entire failure to void urine, may be due either to complete suppression of the secretion in the kidneys or to obstructions in the urinary tract.

Hydruria.—This is a state of the urine in which the fluid is increased out of proportion to the solids, and is usually associated with an increase in the total twenty-four hours' output.

Polyuria.—This term is applied to an increase in the elimination of the urine as a whole, both fluids and solids.

Oliguria is applied to a diminution in the total excretion of the urine.

Polyuria may be noted in the following conditions: 1. Diabetes mellitus. 2. Diabetes insipidis. 3. Chronic interstitial nephritis. 4. Amyloid disease of the kidney.

Oliguria is met under the following circumstances: 1. Valvular heart-disease. 2. Diminished blood-pressure (see section on blood-pressure). 3. Acute articular rheumatism. 4. Chronic parenchymatous nephritis. 5. Acute congestion and

inflammation of the kidneys. 6. Decreased cell activity in shock. 7. Failure of nutrition preceding death.

SPECIFIC GRAVITY.

The average specific gravity of normal urine of 1500 centimeters (fifty ounces) volume for twenty-four hours, is about 1020. Slight variations (1015 to 1028) from this standard are consistent with perfect health and depend chiefly upon the character of the food ingested, the quantity of water taken, and upon the state of metabolism. Under certain conditions of apparent health, as after a hearty meal, the specific gravity of the individual specimen may be as high as 1035, and after excessive ingestion of fluids it may temporarily fall to 1005.

Under pathologic conditions the specific gravity may vary between 1001 and 1055 or even higher. If the diet consists largely of nitrogenous food it will furnish a relatively larger amount of solids; in consequence the specific gravity of the urine will be increased. Active muscular exertion also tends to raise the specific gravity by increasing tissue catabolism. Copious diaphoresis may bring about a concentration of the urine by diminishing the amount. Fasting has a similar effect.

The specific gravity usually varies inversely with the volume both under normal and pathologic condition. A scanty urine is more concentrated than a profuse one. Diabetes mellitus forms an exception to this rule, since the presence of sugar produces a high specific gravity in spite of the excessive amount of the excretion. This fact is so characteristic that a tentative diagnosis may be made upon this alone.

From the foregoing it is evident that, from a clinical standpoint, it is necessary to consider the specific gravity, the total solids, and the total volume together, as they are intimately and logically related to each other, and that the determination of the specific gravity is of greatest value and significance when the total volume of the urine for twenty-four hours is known. Also that the chief clinical value of the study of the amount and of the specific gravity is to aid in the estimation of the total solids of the urine (see below).

The specific gravity of the urine is usually determined with

the aid of a hydrometer or urinometer, but is more accurately determined by the Westphal balance. Only approximately correct results may be obtained with the urinometer.

The Use of the Urinometer.—The scale of the urinometer is usually marked in regular intervals from 1000 to 1060 (Fig. 20). To insure ease and accuracy in reading, these markings should not be too close together. Many urinometers are inaccurately made, so that before purchasing an instrument it is always well to compare it with a standard instrument, or at least to ascertain that it floats at the 1000 mark in distilled water at the standard temperature (15° C. or 60° F.). Although a

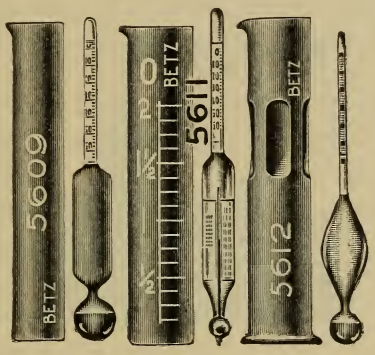

FIG. 20.—VARIOUS FORMS OF URINOMETERS AND URINOMETER CYLINDERS.

large instrument is more accurate, a small one, requiring less urine, is more convenient and must frequently be employed for lack of sufficient urine in which to float the larger instrument.

To determine the specific gravity of the urine: After allowing the urine to cool to room temperature (about 60° F.), it is poured into the urinometer tube (the urinometer and an appropriately sized glass cylinder are usually sold together), and the urinometer immersed in the urine; then with the eye on a level with the surface of the urine, the division of the scale is read off which corresponds to the lowest part of the curve of the meniscus. To insure accuracy the containing cylinder should be sufficiently large to allow the urinometer to float freely and not come in contact with the sides. All bubbles and froth should

be removed from the surface of the fluid by means of filter-paper. If the urine is cooler than 15° C., one-third of a urinometer unit should be subtracted for every degree centigrade below the standard temperature. If warmer an appropriate addition should should be made. Since the specific gravity of individual urine specimens vary greatly during twenty-four hours, it is necessary that the specific gravity should be taken from a mixed specimen of the twenty-four hours' collection. A reading taken from any single specimen is of little clinical significance.

If the specimen is too small to work with in the ordinary way, it is a very simple matter to dilute with a known proportion of distilled water. Estimate the specific gravity of the

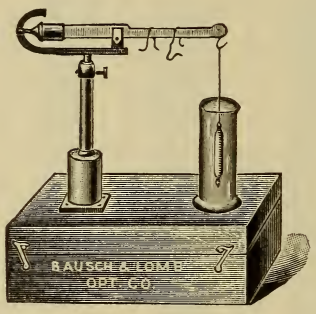

Fig. 21.—Westphal Balance. (A. H. Thomas.)

diluted urine and then calculate the specific gravity of the specimen at least approximately.

The Westphal balance (see Fig. 21) is an extremely accurate method of determining the specific gravity, carrying the reading to the fifth figure (fourth decimal). The method of employing the Westphal balance is as follows:—

When the instrument is mounted the glass plummet which is suspended from one end of the beam will balance in distilled water at 15° C. and represents 1. Now pour the urine into the jar until the twist in the platinum wire is below the surface. Weighing is accomplished as follows: Place the first rider upon the end of the beam above the float, then place the second rider in the first notch to the left on the scale on the beam. If the plummet rises place the rider on the second notch. If now the

beam balances and the temperature of the urine is 15° C., the specific gravity of the urine is exactly 1020. If the float still rises take the third rider and find the notch in which the beam balances or nearly so. If the beam balances with the third rider in the forth notch, the specific gravity is exactly 1024. Should, however, the float still slightly rise, take the fourth rider and find the exact balance, and if this is in the sixth notch the specific gravity is exactly 10246. In other words, the second rider (in size) gives the third figure, the third rider the fourth (third decimal), and the fourth rider the fifth figure or the fourth decimal. With a little practice determinations with the Westphal balance are rapidly and accurately made. Care should be exercised to see that no air bubbles become attached to the cord or float, and that temperature corrections are made as directed below.

Corrections for Temperature.—The temperature of the urine immediately after being voided, ranges from 85° to 95° F. (29.5° to 35° C.); therefore in taking the specific gravity of fresh urine its temperature must be observed, and for every seven degrees Fahrenheit that the thermometer indicates above the temperature at which the instrument is standardized, one degree should be added to the specific gravity, as indicated by the instrument.

METHOD OF ESTIMATING THE TOTAL SOLIDS.

The *accurate chemical method* of weighing is too difficult and tedious for general clinical work, descriptions of which may be found in works on physiologic chemistry.

The normal average weight of solids is sixty-five grams. The specific gravity is a pretty accurate index of the amount of solids excreted by the urine when this has been determined from the twenty-four hours' urine.

The solids excreted in one litre of urine may be approximated in grains by multiplying the last two figures of the specific gravity by 2.2337 (Vierodt's factor).

Trapp's Formula.—Multiply the last two figures of the specific gravity by two, and the result will represent the parts of solids in one litre of urine. Example: If the specific gravity

is 1023, then 23 times 2 will equal 46 parts of solids per 1000 cubic centimeters.

Bird's Formula.—The last two figures of the specific gravity about represent the grains of solids in a fluidounce of urine tested. Thus, a specific gravity of 1022 would contain approximately twenty-two grains of solids per ounce of urine.

Metz's Formula.—Multiply the last two figures of the specific gravity by .00233, and multiply this product by the total twenty-four hours' volume in cubic centimeters. The final product will be the total weight of solids expressed in grams. Example: Specific gravity = 1024: then 24 x .00233 x 1500 cubic centimeters equals 87.27 grams of solids in twenty-four hours' excretion.

THE REACTION.

The reaction of the twenty-four hours' urine is usually acid, sometimes amphoteric, and rarely alkaline. The normal acidity is not due to the presence of free acids, but to acid salts, chiefly acid phosphates. Primarily the reaction of the urine depends upon the character of the diet. Increase in ingestion of foodstuffs containing alkaline salts (vegetables), or when such salts are formed within the body from organic acids contained in the food (fruits), will tend toward an alkaline reaction when the urine is voided. The elimination of alkaline urine in this instance is due to the presence of a fixed alkaline, and therefore cannot be considered pathologic.

It has already been stated that the acid reaction of urine is normally due chiefly to the acid phosphates. Besides these a certain amount of the neutral phosphates of calcium, ammonium, and of sodium usually occur, and it may happen that the acid and neutral phosphates are present in such proportions that the sample will turn blue litmus red and red litmus blue. Such a urine is said to be *amphoteric.*

Urine, when allowed to remain exposed to the air at room-temperature, gradually undergoes ammoniacal fermentation. This is due chiefly to the action of certain micro-organisms upon urea, which is decomposed by a hydrolytic process into ammonia, carbon dioxid, and water (see page 162).

The result of this development of an alkaline reaction is that the soluble phosphates of the alkaline earths are precipitated as tri-calcium phosphate and ammonio-magnesium phosphate, at the same time the soluble urates are transformed into the insoluble ammonium salt (see Fig. 23).

Test.—In order to determine whether the alkalinity of a given specimen is due to a fixed or to a volatile alkali (ammonia), a strip of red litmus is clamped into the cork of the bottle so that it does not touch the liquid but remains dry. Volatile alkali will gradually turn the paper blue, while a fixed alkali will not affect the paper unless the alkaline fluid touches it.

In specimens where the degree of akalinity or acidity is so slight that the litmus-paper test leaves the examiner in doubt, the uncertainty may be eliminated by the following expedient: Take two pieces of litmus-paper, one red and the other blue; allow the urine to come in contact with but half of each strip of paper; then with distilled water allow the whole extent of both strips to become wet. This eliminates any change in the paper due to the presence of moisture alone, and brings out sharply slight changes in the color of the litmus-paper by allowing comparison between the adjacent portions of each strip of paper.

Increased Acidity.—The urine is frequently found hyperacid in:—

1. Fevers.
2. Inflammations of the liver.
3. Hyperchlorhydria.
4. Acute rheumatism.
5. Lithemia.
6. Neurasthenia.

Alkaline urine may be passed under the following conditions:—

1. Undue retention of urine in the bladder.
2. When there is residual urine.
3. Presence of urea-decomposing bacteria in the bladder.
4. Chlorosis.
5. Organic nervous diseases.
6. In marked degrees of general debility.

ESTIMATION OF ACIDITY OR ALKALINITY OF THE URINE.

Reagents.—(a) A one-tenth normal solution of NaOH (1 cubic centimeter equals .004 gram NaOH).

(b) A one-tenth normal solution of HCl (1 cubic centimeter equals .00365 gram HCl).

Since these solutions are difficult and tedious to prepare, it may be well to obtain them ready-made from reliable chemical-supply houses.

The Test.—If the urine has an acid reaction, measure 100 cubic centimeters into a beaker, and add a few drops of a litmus solution. Now add from a burette, drop by drop, sufficient of the NaOH solution to produce an alkaline reaction. State the acidity in this wise: 100 cubic centimeters of urine requires "X" cubic centimeters of one-tenth normal NaOH to render alkaline to litmus. Put in place of "X" the number of cubic centimeters of NaOH used in the examination.

If the urine to be tested is alkaline, then first add to the 100 cubic centimeters exactly 20 cubic centimeters of the one-tenth normal HCl solution. Stir well, and then proceed as in case of the acid urine. State the result as follows: 100 cubic centimeters of urine requires "Y" centimeters of one-tenth normal HCl to render alkaline to litmus. Put in place of "Y" the difference between 20 cubic centimeters and the number of cubic centimeters of NaOH used.

CHEMICAL COMPOSITION OF THE URINE.

A general idea of the chemical composition of the urine and of the quantitative variation of the individual components, may be had from the accompanying table (after Simon). The individuals from whom the urine was obtained were adults. Their habits, diet, etc., may be taken to be that of the average American city dweller.

It must be borne in mind that tabes of Chemical composition are based upon averages taken from a large number of complete analyses, the composition of which, even in perfect health, cover a greater range of variation than indicated in the following table:

CHEMICAL ANALYSIS OF THE URINE (Simon).

	Grams.		
Water	1200	to	1700
Solids	60		
Inorganic solids	25.0	to	26.0
Sulphuric acid (H_2SO_4)	2.0	to	2.5
Phosphoric acid (P_2O_5)	2.5	to	3.5
Chlorine (NaCl)	10.0	to	15.0
Potassium (K_2O)	3.3		
Calcium (CaO)	0.2	to	0.4
Magnesium (MgO)	0.5		
Ammonia (NH_3)	0.7		
Fluorids, nitrates, etc.	0.2		
Organic solids	20.0	to	35.0
Urea	20.0	to	30.0
Uric acid	0.2	to	1.0
Xanthin bases	1.0		
Creatinin	0.05	to	0.06
Oxalic acid	0.05		
Conjugate sulphates	0.12	to	0.25
Hippuric acid	0.65	to	0.7
Volatile fatty acids	0.05		
Other organic solids	2.5		

THE INORGANIC CONSTITUENTS OF THE URINE.

The inorganic constituents of the urine represent the excess of mineral salts that find their way into the blood from the digestive tract or which develop within the body during the processes of metabolism, especially during albumin decomposition. We therefore find that exercise and the ingestion of large amounts of food, as well as the increased cell activity occurring in acute fevers, lead to an increased elimination of salts, and conversely smaller amounts of salts are eliminated when the intake of food in general is restricted. These statements apply particularly to the phosphates.

The bases which are found in the urine in combination with hydrochloric acid, phosphoric acid, and sulphuric acid, are chiefly sodium, potassium, calcium, magnesium, and ammonium. It is believed that the mono-acid phosphates of the alkaline earths are held in solution by sodium chlorid, and also by the di-acid sodium phosphates, to which latter the acidity of the urine is largely due.

While the greater portion of the sulphuric which results

from albumin decomposition is found in the urine combined with inorganic bases, a variable fraction also occurs united with certain aromatic substances which are developed in the intestines during putrefaction and decomposition. The resulting bodies are spoken of as the ethereal or conjugate sulphates. They comprise the alkaline salts of indol, skatol, and phenol.

The Phosphates.—The amount of phosphoric acid excreted by the healthy individual in twenty-four hours ranges from 2.3 to 3.5 grams, the average being 2.8. In the urine the phosphoric acid is found in part combined with the alkaline earths—earthy phosphates—and in part with the alkalies as the alkaline phosphates. The alkaline bases represent about two-thirds of the total phosphoric acid in combination. The earthy phosphates are insoluble in water, but are soluble in dilute acids. They comprise the phosphates of calcium and magnesium. The calcium phosphates predominate. In acid urine the earthy phosphates are in solution, while in alkaline urine they are precipitated. They are thrown down by heat, and also when the reaction of the urine becomes alkaline, whether from a course of internal alkaline medication or from the putrefaction of the urea of the urine. If, during decomposition of the urine, the contained acid phosphates are acted upon by the ammonia resulting from urea decomposition, ammonium-magnesium phosphate (triple phosphate) is formed and appears in the urine as the characteristic prismatic or coffin-lid crystals.

The alkaline phosphates comprise the phosphates of sodium and potassium; of these the sodium is the more abundant. These, unlike the earthy phosphates, are easily soluble in water and in alkaline fluids. The alkaline phosphates form the chief bulk of urinary phosphates. The normal urinary acidity depends upon the presence of these acid phosphates, and not upon the presence of free acid.

While the bulk of the phosphates in the urine is derived from the decomposition of food-stuffs, a part also is derived from the breaking down of the highly complex organic bodies: lecithin and nuclein.

Clinically, the execretion of phosphoric acid and its determination is of very little significance, since it is so largely dependent upon the influence of diet, exercise, etc.

Detection.—If neutral or alkaline urine is heated in a test-tube, a precipitate will be formed which will be found to consist of earthy phosphates. Such a precipitate might be caused by the presence of albumin. To remove the doubt add a few drops of dilute (10 per cent.) acetic acid, when a precipitate due the phosphates will immediately disappear, while if albuminous the cloud will remain or may be increased.

Test for Earthy Phosphates.—To a few cubic centimeters of urine in a test-tube add a few drops of liquor potassii and boil. The earthy phosphates will be thrown out of solution, and may, after settling, be collected upon a filter. Now, to the filtrate add one-third of its volume of "magnesia mixture" (for formula see Appendix). The precipitate thus formed represents the phosphoric acid that was in combination with the alkaline bases, combined now in the form of ammonium-magnesium phosphate.

Estimation of the Phosphates by the Centrifuge.—In a graduated percentage centrifuge tube (see Fig. 30) mix 10 cubic centimeters of urine with 5 cubic centimeters of "magnesia mixture"; invert several times to thoroughly mingle. Revolve in the centrifuge (Figs. 26, 27 and 28) for three minutes. Read off every one-tenth cubic centimeter of precipitate as 1 per cent. by bulk of total phosphates. The average normal percentage by this method is eight. Roughly, each one-tenth cubic centimeter of sediment is equal to about 0.0225 per cent. by weight of $P_2 O_5$.

Determination of the Total Phosphoric Acid.—For this determination the following solutions are required:—

1. A standard solution of uranium nitrate: 20.3 grams of uranium nitrate are dissolved in 1000 cubic centimeters of distilled water, then each cubic centimeter of this mixture is equivalent to 5 milligrams of phosphoric acid.

2. Sodium acetate solution: 100 grams of sodium acetate are dissolved in 900 cubic centimeters of distilled water, and to this 100 cubic centimeters of acetic acid are added.

3. Saturated solution of potassium ferrocyanide.

Method.—Fifty cubic centimeters of urine are placed in a beaker and 5 cubic centimeters of the sodium acetate solution added. The mixture is warmed over a water-bath and uranium

acetate solution added from a burette as long as a precipitate is formed. If the formation of precipitate is not easily recognized, a drop of the potassium ferrocyanide solution may be added; then, as long as a brown color does not appear where the drop falls, the uranium nitrate solution should continue to be added. The end point in the precipitation reaction is reached when a reddish-brown discoloration appears upon the addition of a drop of the uranium nitrate. The quantity of uranium nitrate solution employed to accomplish complete precipitation is now read off from the scale on the burette, each cubic centimeter of which will equal 5 milligrams of phosphoric acid. This number and the 50 cubic centimeters of urine employed furnished the working bases for calculating the percentage.

The presence of sugar or albumin does not interfere with this reaction.

Separate Estimation of the Earthy and Alkaline Phosphates.—Two hundred cubic centimeters of urine in a beaker are rendered alkaline by the addition of ammonium hydroxid, and set aside for a few hours. The earthy phosphates are thus precipitated and may be collected upon a filter-paper, and after washing with dilute ammonia (1:3) are transferred to a beaker, where they are dissolved with as little acetic acid as possible. Distilled water is then added so as to make the total volume approximately 50 cubic centimeters, when the solution is boiled and then titrated as above. In a second portion of urine the total phosphates are determined as outlined above. Then the difference between the two results will represent the quantity of phosphoric acid present in combination with the alkalies.

Significance.—As has been shown above, the phosphates are dependent upon many uncertain factors which are determined with difficulty and are of little clinical significance. When in a given case the phosphates are constantly thrown out of solution, it may be taken as an indication that the formation of gravel or calculus is impending. If the usual signs of ammoniacal fermentation are present, the significance is plain.

THE SULPHATES.

General Considerations.—The major portion of the sulphates appearing in the urine are derived from the food, and comprise the simple mineral sulphates of sodium and potassium. Only a small portion exists in organic combination as the ethereal or conjugate sulphates.

The three predominating conjugate sulphates are: Phenol potassium sulphate, indoxyl potassium sulphate (indican), and skatoxyl potassium sulphate.

The mineral sulphates comprise about nine-tenths of the total sulphates in the urine.

Test for Mineral or Preformed Sulphates.—Add a few drops of acetic acid (to prevent the formation of barium phosphate) to a test-tube half full of urine. Now, upon the addition of a solution of barium chlorid a white precipitate of insoluble barium sulphate will be formed. This precipitate varies in density from a faint white cloud to a bulk that gives a thick creamy consistence to the whole mixture. One can roughly determine by the amount of precipitate as compared with the known normal standard, whether the sulphates are increased or not.

Test for Conjugate or Ethereal Sulphates.—Mix equal quantities of alkaline barium chlorid (see Appendix) and urine in a test-tube. After allowing a few minutes for the precipitate to form the mixture is filtered. This process precipitates both phosphates and preformed sulphates. The filtrate is now acidified with 5 cubic centimeters of a (one-fifth vol.) HCl solution and then boiled for some time. The presence of ethereal sulphates is indicated by a reddish discoloration of the fluid which in addition becomes turbid.

Potassium Indoxyl Sulphate or Indican.—As this substance represents the characteristic ethereal sulphate, it may be taken as an indicator for the whole group, and the tests for this substance used for estimating the relative amount of the whole group of ethereal sulphates in the urine. The several tests for indican are based upon the fact that an excess of HCl will liberate the indoxyl, which can then, by the addition of an oxidizing agent, be converted into indigo-blue, and finally this can be recognized in small amounts by extraction from the bulk of urine with chloroform.

Test for Indican (modified Jaffee).—Take 20 cubic centimeters of filtered urine in a test-tube and add 3 or 4 cubic centimeters of chloroform, and one drop of a 1 per cent. solution of potassium chlorate, and finally 20 cubic centimeters of HCl. This mixture is to be thoroughly agitated and mixed by pouring repeatedly from one test-tube to another. This should be repeated at intervals of two or three minutes covering a period of ten minutes. The presence of indican is indicated by a blue discoloration of the chloroform. In the presence of a large amount of indican the chloroform will appear almost black, while the whole mixture will assume a dusky bluish-red color. (For comparative color-scale see Plate III.)

While a trace of indican cannot be considered pathologic, there are, nevertheless, many specimens, possibly one-third, which fail to show any discoloration of the chloroform.

Caution.—An excess of oxidizing agent, either in volume or strength, will prevent a positive reaction through over-oxidation of the indoxyl compound. 2. An excess of chloroform will result in too great dilution of the indigo, causing failure to detect traces of indican. 3. A reddish discoloration of the chloroform is not due to indican. Such a reaction occurs in the urine of patients who are taking iodide, and possible bromide. The simultaneous occurrence of a red and a blue reaction, will produce a color bordering on purple. This possible source of error may be removed by the addition of a few drops of a 10-per-cent. solution of sodium thiosulphite, which will bleach to pink color due to iodine.

Clinical Significance of Indican.—The presence of more than a trace of indican probably denotes the existence of undesirable decomposition in the intestinal tract. The occurrence of a positive indican reaction does not necessarily indicate constipation.

THE CHLORIDS.

The quantity of chlorids in the urine is usually decreased in: 1. Most febrile diseases. 2. Nephritis. 3. Wasting diseases. 4. Pneumonia.

Approximate Estimation of the Chlorids.—To a few cubic centimeters of urine in a test-tube, add a few drops of nitric

PLATE III.

Iodine Reaction
Indican Absent
Very Faint Trace
Trace
Large Trace
Moderate Amount
Large Amount
Very Large Amount

acid, boil and filter to remove the albumin. Add to the filtrate a few drops of a 10-per-cent. solution of silver nitrate. The abundance of the white cloud will roughly indicate the amount of chlorids present.

Accurate Methods of Quantitative Determination.—Method of Salkowski-Volhard. Take 10 cubic centimeters of urine in a beaker and dilute with 50 cubic centimeters of distilled water, and then treat with 4 cubic centimeters of concentrated nitric acid and 15 cubic centimeters of a standard silver nitrate solution. The mixture is then further diluted with distilled water up to 100 cubic centimeters, and after thorough agitation is passed through a dry filter. In a carefully measured portion of the filtrate the excess of silver is carefully titrated with a solution of potassium sulphocyanide of such a strength that 25 cubic centimeters corresponds to 10 cubic centimeters of the standard silver solution. A few drops of a saturated solution of ammonio-ferric alum serve as an indicator. The amount of silver solution employed to precipitate the chlorids in the 10 cubic centimeters of urine is then calculated. The number of cubic centimeters necessary for this precipitation is multiplied by 0.01, which will indicate the amount of chlorids in 10 cubic centimeters of the urine calculated as sodium chlorid. The presence of albumins or of sugar does not interfere with this reaction.

THE ORGANIC CONSTITUENTS OF THE URINE.

GENERAL CONSIDERATIONS.

The organic constituents of the urine comprise the normal end-products of nitrogenous metabolism within the body, also various products of albuminous putrefaction which have found their way into the general circulation from the intestinal tract. Finally certain pigments which bear a relation to the normal blood-pigment, and various substances of obscure origin are encountered. Under abnormal conditions we may meet with normal constituents of the blood which do not ordinarily appear in the urine. Lastly, in pathologic conditions, we meet various products of abnormal metabolism.

UREA.

Urea was first synthetically prepared from ammonium cyanate in 1828 by Wohler. Formerly it was supposed that urea resulted from uric acid through a process of oxidation, and that this was its only source. Now this error is well known, and while it is recognized that the formation of urea from uric acid is possible and that a small portion of the total amount may be derived in this manner, modern research has shown that in man urea is largely derived from the destruction of the nucleins within the body, and that the sources of the nucleins are both the tissue cells and the cells of animal foods ingested.

It has been repeatedly shown that during the decomposition of albumins by means of acids and alkalies, as during the process of tryptic digestion and albuminous decomposition, a large amount of mono-amido-acids results. And it is supposed that these bodies probably represent the intermediary products in the transformation of albuminous nitrogen into urea. Under certain pathologic conditions these acids may appear in the urine, and when they are there noted the elimination of urea is much diminished. In health, however, this does not occur, and on examination of the different tissues of the body such acids are found only in traces. We must conclude, therefore, that these acids, supposing them to occur as the primary products of albuminous decomposition within the body, are transformed at once into other substances, which in turn give rise to urea.

It has also been shown that the amido acids yield carbamic acid on oxidation, and that on alternate oxidation and reduction urea can be produced from the ammonium salt (ammonium carbamide). While it is generally assumed that urea is largely referable to a transformation of the mono-amido-acids into ammonium carbamate, and while it has also been shown that such a transformation does actually occur, it must be remembered that at best only traces of these amido acids are found in the tissues.

From these conclusions it is reasonable to believe that the greater portion of albuminous nitrogen is set free from the various organs of the body in the form of an ammonium salt of paralactic acid, and that this salt is now generally conceded to be the antecedent of urea.

It is probable that a certain amount of urea is produced in the body in a number of ways, and there is ground for the belief that its formation is not confined to a single organ. The greater part is, without doubt, produced in the liver. In corroboration of this fact it has been repeatedly shown that in disease of this organ, when accompanied by extensive destruction of the glandular elements, a diminished amount of urea is found in the urine, while ammonia and lactic acid are found in increased amounts. In certain cases of this class as much as 37 per cent. of the total urinary nitrogen has been found in the urine in the form of ammonia.

If we accept the modern doctrine that urea originates not only in different ways, but that it may also be formed in other organs beside the liver, then we can understand why it is that in certain diseases of the liver the diminution in the amount of urea excretion is not always proportionate to the extent of parenchymatous degeneration, and that no case has yet been reported in which the excretion of urea has ceased altogether.

Nitrogenous Equilibrium.—The albumins are the ultimate source of the urea, and according to Pettenkofer they exist within the body in two forms, viz.: as organized albumin, which is built up into tissues, and the so-called circulating albumin, which is present in excess of the actual requirements of the body and which may be broken down directly and eliminated in the urine without ever having entered into the construction of the body-proper. This latter portion of the body albumin is said to furnish the bulk of the urea, while the fixed tissue albumin represents the minor but more uniform source. The actual amount eliminated will, therefore, be primarily dependent upon the quantity ingested.

Experiment has shown that under normal conditions of average diet the total urinary nitrogen is practically equivalent to the quantity ingested, barring a small fraction, which escapes digestion and appears in the feces. Such a condition is spoken of as the nitrogenous equilibrium of the body. Of this relation infinite variations exist, which may even vary from time to time in the same individual. If the amount of nitrogenous food is suddenly diminished the amount of urinary nitrogen will also decrease; then if the reduced ingestion remains constant the

nitrogen output, while lowered, will at the same time tend to remain level. If, on the other hand, the nitrogen intake is increased, an increased nitrogen elimination speedily follows, but here a certain fraction will be retained in the body and gradually a higher level of equilibrium will be established.

There are natural limits to this power of accommodation of the system to nitrogenous ingestion and elimination, so that we may find a point which varies in different individuals where a further nitrogen ingestion does not lead to a higher level of nitrogenous equilibrium, and when, consequently, a further retention of nitrogen does not occur. Overfeeding then results in various digestive disturbances. Diarrhea and vomiting may occur through nature's effort to protect the body from a further increase in circulating nitrogen.

Underfeeding, on the other hand, generally leads to increased destruction of the organized albumins. Although for a while the body's store of fats and carbohydrates is capable of protecting the body against an undue loss of nitrogen in this direction, still if the reduced intake of nitrogen continues sufficiently long, death finally ensues.

From the fact that the level of nitrogenous equilibrium varies in different individuals and in the same individual from time to time, it follows that the amount of urea excreted must also vary according to the same irregular manner. Any figures, therefore, which are supposed to indicate the amount of urea eliminated, can be of little and uncertain value unless the actual state of the individual's health is known, also his body-weight, habits as to exercise, the amount of nitrogenous food ingested, etc. Only when we are in possession of an accurate knowledge of these several factors can we say whether the urea excretion is or is not normal.

Certain figures have been compiled by physiologists to indicate the amount of nitrogen which should enter into the composition of the diet and from which we may approximately calculate the amount of urea that should be excreted. By estimating this, or still better, by determining the total nitrogen elimination, we can then determine whether or not the individual is consuming the proper amount of nitrogenous food in his dietary. While such figures may serve as a general guide,

they have mostly been constructed without due regard for the factors above indicated, and should not, therefore, be too implicitly relied upon.

According to Simon, among the well-to-do classes the elimination of from twenty to twenty-five grams of nitrogen in twenty-four hours is about normal, taking the average body-weight of the individual into consideration. A smaller amount is not infrequently met in persons of sedentary habits who may appear to be in perfect health.

Urea in Disease.—While extensive variations occur in the urea excretion of health, still greater variations from the average standard are noted in disease. But here also should be taken into account the amount of nitrogen ingested in relation to the body-weight.

An increased elimination of urea referable to the destruction of organized albumins is frequently observed, but this may in cases be obscured by a deficient nitrogen ingestion unless the total intake of the latter is not definitely known. It is of interest here to note that in certain diseases of the liver in which there is great destruction in the parenchyma, the amount of urea may be markedly diminished, even when a fairly abundant supply of nitrogen is taken in. This will be found due largely to the interference in urea synthesis, and as a secondary result we find that in these cases a considerable portion of the nitrogen appears in the urine in the form of ammonium salts of paralactic acid (see page 182) and of carbamic acid; in extreme cases as mono-amido-acids and as leucin and tyrosin.

Properties of Urea.—Urea crystallizes in colorless, quadrilateral, or six-sided prisms with oblique ends, or when rapidly crystallized in delicate white needles which melt at 132° C., but which are probably decomposed at a temperature of 100° C. They contain no water of crystallization, and are permanent in the air, and easily soluble in cold water, in which they form a neutral solution. With nitric acid, urea unites to form urea nitrate, which crystallizes out in octahedral lozenge-shaped or hexagonal platelets. Urea nitrate is readily soluble in distilled water, but is soluble less readily in water acidified with nitric acid. Its formation is frequently observed when urine is examined cold, with nitric acid, for the presence of albumin. On

heating, the crystals are decomposed without leaving any residue.

Detection of Urea.—1. To detect urea place a drop or two of the suspected fluid upon a glass slide, and after adding a drop or two of nitric acid, warm gently. If urea is present, after partial evaporation and cooling, the microscope will show the characteristic crystals of urea nitrate. These are either rhombic or hexagonal plates, frequently overlapping like shingles on a roof. Their acute angles measure 82°.

2. Add to the suspected fluid, in a test-tube, a few drops of fresh sodium hypobromite. A rapid evolution of gas will indicate the presence of urea.

Since clinical observations are concerned in the total output of urea, it becomes necessary to estimate the quantity or percentage from a sample of twenty-four hours' urine.

Under normal conditions of average health the percentage of urea is two.

The average daily excretion of urea is forty grams or five hundred grains, or about half the weight of the total solids.

Quantitative Estimation of Urea.—This quantitative estimation is determined by calculation from the observed volume of nitrogen gas evolved from a measured quantity of urine by a process of decomposition. For practical purposes one gram of urea is estimated to furnish 37 cubic centimeters of nitrogen gas. The decomposition of the urea contained in the measured volume of urine is accomplished by means of an alkaline solution of sodium hypobromite (see Appendix for formula of Knop's solution). The test is best conducted in a Doremus-Hinds ureameter (see Fig. 22).

The Test.—First pass some urine through the small tube to wet the stop-cock, then fill the large tube with Knop's solution, and the smaller side tube with urine exactly up to the 0 cubic centimeter mark. Now, drop by drop, allow exactly 1 cubic centimeter of urine to pass into the reagent in the large tube. When the bubbles of gas (nitrogen) cease to rise, the fraction of a gram of urea may be read directly from the graduated scale on the larger tube.

Example.—Suppose that after admitting exactly 1 cubic centimeter of urine the level of the fluid stands at the 0.018

mark. Then the 1 cubic centimeter of the sample contained 0.018 gram of urea. To obtain the total amount of urea excreted in twenty-four hours, it is only necessary to multiply this figure by the number of cubic centimeters in twenty-four hours' urine.

URIC ACID.

General Considerations.—Uric acid, like urea, is nitrogenous. The normal proportion of uric acid to urea is as 1 : 45. In health it exists in solution as sodium and as potassium urate. A healthy adult excretes 0.2 to 1.0 gram of uric acid in the course of twenty-four hours. The amount increases physiologically

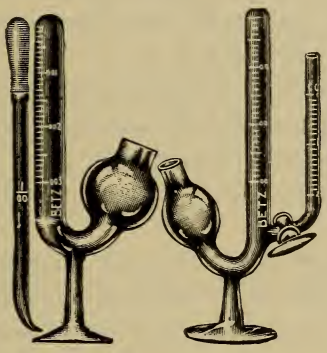

FIG. 22.—VARIOUS FORMS OF UREAMETERS.

with increased ingestion of food, and pathologically with increased nitrogen metabolism in about the same proportions as urea. The amount of uric acid in urine varies directly with the specific gravity, so that the last two figures of the specific gravity (calculated to four places), multiplied by two, give approximately the number of centigrams of uric acid in the litre. The daily excretion of uric acid is increased in fevers and in leukemia. The relation of the elimination of uric acid to attacks of gout and the so-called uric acid diathesis, is still an unsettled question. Of necessity the elimination of uric acid is increased by the ingestion of uric acid and other purin bodies, as well as by foods rich in nuclein (rich in cells).

Properties of Uric Acid.—Uric acid is practically insoluble in cold water, requiring 18,000 parts of water to dissolve 1 part

of uric acid. It is freely soluble in alkalies and in solutions of the carbonates. Pure uric acid crystals are colorless, transparent platelets, the angles of which are frequently irregular and rounded off. Such crystals are occasionally seen in freshly voided urines, but more commonly they are found in the form of brownish-yellow, whetstone-shaped crystals, occurring singly or in groups (see Plate IV, *a* and *b*). Uric acid once deposited remains undissolved in acid urine. Owing to the inclusion of the urinary pigments in the crystals, these, when present in appreciable quantity, are spoken of as "brick-dust."

Microscopic Appearance.—The reddish specks observed by the naked eye are found, upon microscopic examination, to be various modifications of rhombic prisms (see Plate IV, *c, d, e* and *f*). The simpler forms have some resemblance to the conventional lozenge or whetstone.

Significance.—If these crystals deposit in appreciable quantities soon after micturition, it may be considered as a sign of impending gout or gravel formation. Such a deposit, however, is not necessarily evidence that the elimination of uric acid is excessive.

Isolation of Uric Acid.—To 200 cubic centimeters of urine add 10 cubic centimeters of HCl, and let stand for twenty-four hours; the uric acid will then have settled to the bottom of the container, from which it may be collected by decanting, filtering, and finally washing in cold water.

Qualitative Tests for Uric Acid.—Murexid Test: Put the solution supposed to contain uric acid or urates in a porcelain dish, add a drop of nitric acid and evaporate to dryness. After cooling, allow a drop or two of ammonia water to come in contact with the residue. The presence of uric acid or urates will be shown by bright blue or violet (murexid) color.

Schiff's Test.—Having a residue prepared as above, or crystals supposed to be uric acid, dissolve in a test-tube with the aid of a solution of sodium carbonate. Moisten some filter-paper with a 10-per-cent. solution of silver nitrate. Into the center of this paper allow a few drops of the uric acid sodium carbonate solution to fall. In the presence of uric acid the silver nitrate will be reduced to black metallic silver.

Approximate Quantitative Determination. — According to

Gubler[2] the amount of uric acid in urine may be approximately determined by stratifying the specimen of urine to be tested upon a layer of nitric acid contained in a test-tube, so that the volume of urine to the volume of nitric acid is as 3 : 2. After a short interval uric acid crystals will separate out as a cloudy white ring at the line of junction of the two fluids. If the amount of uric acid in the specimen is increased, the precipitation will be plain in five minutes or less. If diminished it will not appear until later. This determination is of value only when the daily excretion of urine is approximately normal, and if it is diminished in amount it should be diluted with water up to the average amount before applying the test. Obviously the conclusions derived from such a method should be given very slight weight clinically, and then only when the estimation is made with a part of the twenty-four hours' collection, properly diluted as mentioned above. Albumin must first be removed by slight acidulation with dilute acetic acid, boiling and filtering, after which the test should be applied when the urine has cooled to room-temperature.

THE PURIN BASES IN THE URINE.

These comprise xanthin, hypoxanthin, heteroxanthin, paraxanthin, guanin, and adenin. These bases are normally present in the urine and comprise approximately one-tenth as much as the normal uric acid. The amount of purin bases normally found in the urine in twenty-four hours varies between 0.028 and 0.058 gram. There is at present no practical clinical method of estimating them.

THE URATES.

General Considerations.—When the urine cools the urates may settle to the bottom of the container as a cloudy reddish-yellow precipitate. This is most likely to occur when the urine is scanty, concentrated, and highly acid. This condition often obtains in fevers, and in congestion or inflammation of the kid-

[2]Laquer: Schmidt's Jarhbucher. Vol. ccxxxvi, No. 10.

neys. The sediment then presents a fairly characteristic appearance, being clay-colored, reddish-yellow or rose-red. It may adhere to the walls of the vessel as a fine, reddish coating.

The ordinary uratic sediment consists of a mixture of the urates of sodium, potassium, calcium, magnesium, and ammonium. Sodium urate predominates. With the exception of ammonium urate, these urates only appear in acid urine. Uratic sediments often contain a few crystals of uric acid which have been formed during the double rearrangement of molecules which resulted in the precipitation of the urates. If

FIG. 23.—URINARY SEDIMENT.
a, Triple Phosphates. *b*. Ammonium Urate in Alkaline Urine.

urine containing a uratic sediment is decomposed by ammoniacal fermentation, the sediment will be changed to acid-ammonium urate, and this latter is the only urate sediment which occurs in alkaline urine.

If freshly voided urine is kept in a cool place, the precipitation of urates will occur rapidly. This same precipitation occurs at a higher temperature if the amount of urates is in excess of normal or if the urinary acidity is decreased. This precipitate is readily distinguished from phosphates by its prompt disappearance upon the application of heat.

Qualitative Tests.—1. Half fill a test-tube with turbid urine and apply heat to the upper part. If the turbidity is due to the

presence of urates, the heated portion of the urine immdiately becomes clear.

2. To some urine in a test-tube add some liquor potassii, when the turbidity due to urates promptly disappears.

Microscopic Appearance of Urates.—The uratic deposit is composed of fine, somewhat regular granules, usually occurring in groups; sometimes the granules show spiny projections. Ammonium urate occurs only in alkaline urine, and is generally accompanied by a copious precipitation of triple phosphates. Ammonium urate appears as opaque brownish-red spherules with or without projecting spines. (See Fig. 23.)

Significance.—The excess of urates is of no special importance; they are increased in most conditions, accompanied by fever, and in many disturbances of metabolism.

HIPPURIC ACID.

Hippuric acid, in combination with alkaline bases, is a normal constituent of the urine. The average quantity eliminated in twenty-four hours is one gram. This amount may be increased by exercise, by a vegetable diet, and by ingestion of benzoic acid. In cases where the total excretion of urine is greatly diminished, hippuric will be spontaneously thrown out of solution. This is, however, a rare occurrence, because hippuric acid is readily soluble in water.

Microscopic Appearance.—The crystals are characteristic rhombic prisms, resembling in a measure the coffin-lid crystals of triple phosphates. Hippuric acid crystals may, however, be distinguished by the fact that they are precipitated in acid urine only, and also because they do not dissolve on the addition of acetic acid. Phosphates, on the other hand, are precipitated only in neutral or alkaline urine, and are readily soluble in dilute acetic acid.

CREATININ.

This normal urinary constituent is present in the twenty-four hours' specimen to the amount of one gram. Creatinin is derived from the creatin of muscle. It is distinguished in the urine by its union with mercuric chlorid, with which salt it

forms insoluble compounds. Similar characteristic compounds are formed with zinc chlorid and silver nitrate. The zinc chlorid combination has a characteristic appearance, by which this substance may be recognized. Under the microscope the zinc chlorid combination of creatinin appears as minute needles arranged in balls and rosettes. Creatinin reduces alkaline copper solutions, and therefore affects, in a slight degree, the accuracy of the quantitative sugar estimations which depend upon the reducing power of sugar-containing urine.

OXALIC ACID.

Oxalic acid is normally present in the urine only in very small amounts and it appears in combination with calcium as calcium oxalate. The normal amount of this substance in a urine of normal volume is held in solution by the acid sodium phosphate. Since oxalic acid requires 500,000 parts of water to effect solution of 1 part calcium oxalate, even the slightest variation in the normal relation results in its appearance as a deposit. Artificially the crystals may be thrown down by carefully neutralizing the specimen with dilute ammonia water, or by simply allowing the urine to stand for a time exposed to the air. The sediment of calcium oxalate is usually so scant that it is only recognized by the aid of the microscope. The more rapid the formation of these crystals in the urine after voiding, the smaller will the individual crystals appear; this will, in a way, indicate whether or not the presence of the crystals is due to a pathologic increase in the oxalic acid content, or merely to a change of reaction (loss of acidity) from standing. The typical calcium oxalate crystal is a perfect octahedron without color, insoluble in acetic acid, but freely so in hydrochloric acid. (Plate V, *a* and *b*.)

The presence of crystals of the oxalates in the urine does not necessarily imply a pathologic increase in the total quantity of oxalic acid, since an increase in oxalic acid elimination can only be determined by a careful quantitative estimation from the twenty-four hours' specimen. It is probably that "oxaluria" is often diagnosed on insufficient grounds: a too hasty assumption, based on the finding of a few crystals, should be guarded against.

Microscopic Appearance.—Oxalates are recognized in one of two forms: perfect octahedra, or in hour-glass and dumb-bell forms.

Significance.—These deposits sometimes follow the eating of stewed rhubarb or other acid fruits containing oxalates. Usually their existence in freshly-voided urine, indicates imperfect oxidation of retarded metabolism within the economy.

CYSTIN.

This substance, in exceedingly small amounts, is a normal constituent of urine. It is almost insoluble in water, and in the very rare cases where it is present in increased amount, it is deposited. To the naked eye the deposit is abundant and somewhat resembles that of urates. It is, however, not dissolved by heat or by the vegetable acids, but is readily dissolved in ammonia water. The ammoniacal solution exposed on a slide and examined under the microscope, slowly develops crystals in the form of hexagonal plates. (Plate VI, *d*.) Iodoform, which has found its way into the urine from surgical dressings, may be mistaken for cystin.

Tests.—When urine containing cystin undergoes decomposition, it develops the odor of hydrogen sulphide. When boiled with a solution of lead oxid in sodium hydrate, black lead sulphid is formed.

LEUCIN AND TYROSIN.

These are present in the urine in cases of acute yellow atrophy of the liver, typhoid fever, and phosphorus poisoning. (Plate VI, *e* and *f*.)

To examine for these substances, the urine must be concentrated on a water-bath, and the cooled liquid examined under the microscope.

Microscopic Appearance.—Leucin appears as greenish-yellow spheres with concentric markings or radiating spines. Tyrosin as feathery sheaves, which resemble the frayed end of a rope.

PART II.

ABNORMAL CONSTITUENTS OF THE URINE.

ALBUMIN.

General Considerations.—Albumin is probably present in minute quantities in all urine, since urine always contains a variable number of cellular elements derived from the urinary tract. Thus, even under normal conditions, by means of sufficiently delicate tests, we can always demonstrate the presence of albumin. This amount is, however, so small that it returns a negative result with the tests commonly employed in the clinical laboratory for the detection of albumin in urine. Therefore the presence of demonstrable amounts of albumin, by the tests now to be described, must be considered as variations from the normal. The "normal" albumin is *nucleo-albumin,* and its clinical significance is slight, even when present in an appreciable quantity.

Several other albumins are found in the urine, of which *serum-albumin* and *serum-globulin* are of the greatest clinical importance, and the term albuminuria is understood to indicate the presence of one or the other, more often of both, in the urine. Other albumins which may be found in the urine, besides those referred to above, are albumose and fibrin.

The diagnostic and clinical importance of *albumosuria* have not yet been determined. Traces have been found in many of the infectious fevers. A large albumosuria may be significant of multiple myelomata, since in this affection large amounts are frequently observed. It has also been found in osteomalacia and in nephritis.

Fibrin, when present, indicates the direct entrance of blood-plasma into the urinary tract. It must be distinguished from blood-clots, which come under the head of hematuria.

Causes of Transient Albuminurias.—Physiologic or Functional Albuminuria: The presence of easily demonstrable amounts of albumin can hardly be considered normal under any circumstances. However, the presence of a functional albu-

minuria is recognized by some authorities. An albuminuria does, however, occur, in which the organic change in the kidney, if there be any, is so slight and evanescent as to be unimportant. This form of albuminuria, compared with that caused by severe and permanent kidney change, may, with propriety, be termed functional, but never, physiologic.

Functional.—Albuminuria may occur after violent exercise, after a cold bath, after severe mental strain, and after overeating of proteid food-stuffs, particularly eggs.

Whether or not the disturbance giving rise to the albuminuria is insignificant and transient (renal hyperemia or anemia), or partakes of the gradual and progressive variety due to permanent alteration in kidney structure, to which the term chronic nephritis is applied, must be determined by the history, symptoms, and progress of the case.

Fevers.—Here the occurrence of albumin in the urine is, in all probability, dependent largely upon the intensity of the infective process, causing irritation of the kidney epithelium, incident to the passage of toxic substances circulating in the blood, through the glomeruli and tubules of the kidneys; the inflammatory process so induced being indicated by the amount and duration of the albuminuria.

Specific Blood-Changes.—The nature of the changes in the blood occurring in scurvy, peliosis, purpura, hemophilia, and pernicious anemia, is obscure in relation to the production of albuminuria.

Foreign Substances in the Blood.—This form of albuminuria is seen after the excessive ingestion of lead, mercury, ether, chloroform, etc. These probably produce the albuminuria by irritation of the kidney through efforts of that organ at elimination of the substances themselves, or their oxidation products.

Circulatory Disturbances.—Here the passive congestion occurring in cardio-vascular and pulmonary diseases directly affects the permeability of the renal epithelium.

Neurotic.—This term applies to albuminuria occurring during or immediately succeeding attacks of apoplexy, migraine, tetanus, delirium tremens, and certain head injuries. It is probably toxic in origin.

Extra-Renal.—The presence of blood, pus, chyle or lymph, which has entered the urinary tract in appreciable quantities, will cause albumin to appear in the urine. Recently attention has been called to a form of albuminuria resulting from abnormalities in the function of the genital tract. This form comes and goes irregularly, and does not show the presence of spermatozoa.[2]

In the majority of the above conditions the quantity of albumin is small and varies from day to day. Under these circumstances the relative clinical importance of the finding will be determined by microscopic examination of the urine, aided by the history and course of the disease in which it occurs.

These small and transient albuminurias are of considerable importance from the standpoint of life insurance, since too much importance is frequently placed on the finding of a trace of albumin, particularly in a single specimen, which is usually voided succeeding a full meal.

Qualitative Tests for Albumin.—In detailing the following methods for determining the presence of albumin in the urine, no attempt will be made to offer a great variety of tests. The principle involved in the majority is the same, and depends on the coagulation of albumin in the specimen by heat or by chemical reagents.

Before proceeding to make the tests, the urine must be filtered or centrifugated to remove the turbidity. If this is due to large numbers of bacteria it cannot be entirely removed by this process. The persistence of a bacterial turbidity will interfere with the delicacy of the tests.

1. Boiling and Acetic Acid.—This is the best and simplest test for routine laboratory work. If albumin is found it is well to corroborate the finding by another method. The urine should not be alkaline in reaction.

The Test.—Fill a narrow test-tube almost to the top with clear urine, boil the upper third, being careful to avoid too much agitation. In the presence of albumin, or the neutral earthy phosphates, or of both, a white cloud will appear in the heated area. Now, upon the addition from a pipette of a few drops of

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[2] William Glenn Young: New York Med. Jour,. Jan. 5. 1907.

10 per cent. acetic acid, the cloud, if due to phosphates, will vanish, while if caused by albumin it will remain and will perhaps be increased in density. The chief advantages of this method are its simplicity and delicacy, and the fact that it allows a comparison between the treated and untreated urine in the same tube. The test is most satisfactory when performed in strong daylight with the tube held close to a dull-black background.

Caution.—At times the addition of more acid to a urine already acid will convert the albumin into an acid albuminate, which is not coagulable by heat. To overcome this the acetic acid should always be dilute (10 per cent.), and should never be added to acid urine before boiling, and then only in sufficient quantity to produce the desired reaction.

2. Nitric Acid by Contact.—This test is best performed with the Meeker albumin tube.

The Test (with the Meeker tube).—Allow about one-half inch of warm, clear urine to enter the tube through the small curved tip. Place the forefinger over the upper end of the tube to retain the urine, and dry with care the outside of the tube; plunge it immediately into nitric acid of sufficient depth to allow the acid to flow in and elevate the urine above it. If this is done with care the line of demarkation between the urine and the acid will be very sharp, and the presence of albumin will be shown by a white line at the plane of contact. Five minutes should be allowed for the appearance of the reaction, the delicacy of which is increased if both the urine and the acid are previously warmed to about 60° C. (a temperature too high to be endured by the hand for more than a few seconds).

Sources of Error.—If a cloud appears at the line of contact in a urine known to contain an excess of urates, this source of error can be eliminated by diluting the urine with one or two volumes of water, and repeating the test.

Nitric acid precipitates certain resins which appear in the urine after it has been administered for medicinal purposes. If these cannot be excluded another test should be employed. If an excess of phosphates exist they may be held in solution by the addition of a few drops of a 10-per-cent. solution of acetic acid.

Nucleo-albumin may be held in solution by the addition of one-sixth volume of a saturated solution of NaCl, and then proceeding as before.

Purdy's Test.—Mix a half inch of Purdy's reagent (see Appendix) with four inches of urine in a six-inch test-tube; if a whitish cloud appears either at once or after standing, the urine contains albumin.

Tanret's Test.—Acidify a quantity of urine with dilute acetic acid, if mucin produces a cloud, filter and to the filtrate add about 5 cubic centimeters of alcohol and heat slightly. Now substitute this prepared urine for the urine used in the description of the nitric acid contact-test, using Tanret's reagent in place of the nitric acid (see Appendix); the presence of a white line at the level of contact denotes albumin.

Quantitative Estimation of Albumin.—Method of Esbach: For clinical purposes as a ready means of comparing the albumin content in a given case or a number of cases, this method will be found applicable and convenient.

It must be borne in mind that the resulting amount of albumin, which is commonly spoken of as the percentage of albumin, means the percentage by bulk of the moist, gravitated precipitate, and should never be confounded with the actual percentage of albumin as determined by accurate weighing methods. This never amounts to more than 4 or 5 per cent., while by the method of Esbach the moist precipitate may exceed half of the bulk of the fluid in the tube.

The Test.—Fill the albuminometer tube (see Fig. 24) with urine to the line marked "U," and then fill with the Esbach reagent (see Appendix) to the line marked "R." Thoroughly agitate and then stand aside for twenty-four hours. The number on the scale on the glass tube which marks the height of the coagulated albumin represents the percentage of albumin in the urine. To insure accuracy the tube should stand in a temperature of about 15° C. (60° F.), and the specific gravity of the urine should be reduced to between 1006 and 1008 by dilution, the amount of dilution being taken into consideration in calculating the percentage of albumin.

Albumosuria.—Albumose appears in the urine as a part product of proteid metabolism. It is a pre-peptone which ap-

pears in the blood and which can be produced by artificial digestion. Pathologically, its continued and marked presence in the urine usually denotes multiple myelomata. It has also occasionally been noted in syphilis, pneumonia, peritonitis, and cholera.

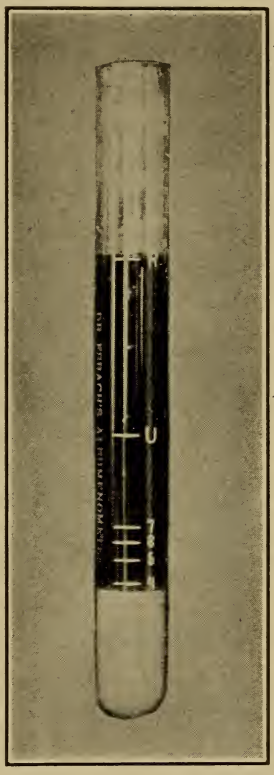


FIG. 24.—ESBACH ALBUMINOMETER.
At end of 24-hour test albumin per cent. reading a fraction less than 4 per cent.

THE TEST.—Add concentrated nitric acid to the urine, agitate and allow to stand in a cool place. A heavy precipitate will occur which, upon heating, disappears, only to reappear on cooling. At the same time the mixture gradually assumes an intense yellow color.

Albumin of Bence Jones.—This substance has been repeatedly found in cases of multiple myelitis. Its exact nature is

not definitely known, but it is generally regarded as an albumose. Its reaction to heat and nitric acid is similar to that of albumose. The test for its positive identification is complicated and difficult, and will hardly be required of the clinical laboratory worker.

Nucleo-Albumin.—Nucleo-albumin, beyond a faint trace, will give the same reactions as true blood-albumins. Its presence may be inferred when there is an albumin reaction in a specimen which shows an absence of renal elements with an excess of epithelia of bladder and urethral type.

Test.—To dilute acid urine add an excess of acetic acid, stand aside, and gradually a faint cloud will appear.

Differential Test.—If the preceding test gives a positive reaction, take a fresh portion of the same specimen, and first add one-sixth volume of a saturated sodium chlorid solution, and then some dilute acetic acid. The presence of sodium chlorid will prevent the precipitation of nucleo-albumin in the last test.

Fibrin, when present in the urine, usually occurs in sufficient amounts to form clots, which are easily distinguishable. When the amount is small it may become necessary to determine the presence of this substance by chemical means.

Test for Serum-Globulin.—Add to some urine contained in a small beaker sufficient ammonia water to produce a slightly alkaline reaction; a cloud of phosphates will appear and will settle to the bottom. As soon as a layer of clear urine appears, decant this into a test-tube. Mix this clear urine with an equal quantity of a clear saturated solution of ammonium sulphate. If a flocculent precipitate appears in a few minutes the albumin present is serum-globulin.

Test for Serum-Albumin.—Take the final solution obtained in the foregoing test and filter it free from serum-globulin. Heat the filtrate to boiling, and add one-tenth volume of strong nitric acid. If a precipitate now appears the urine contains serum-albumin.

Proteose.—Mix about 50 cubic centimeters of urine contained in a beaker with 5 cubic centimeters of strong nitric acid. Heat the solution rapidly to about 80° C. If a precipitate appears, prepare a hot filter-paper by pouring boiling water through it in a funnel. Now filter the hot mixture of

urine and nitric acid. Add to the clear, hot filtrate its own volume of a clear, saturated solution of sodium chlorid. Allow the mixture to cool. If any precipitate appears in this cooled mixture then proteose was present in the specimen of urine under examination.

GLUCOSE.

General Considerations.—A trace of sugar can at times be detected in apparently normal urine from healthy subjects by special tests of great delicacy. The ordinary tests employed in clinical medicine, however, do not react to these minute amounts of sugar, hence the findings of glucose by the tests about to be outlined must, in every case, be considered pathologic.

The reducing power of normal urine is, in part, due to the activity of uric acid and creatinin, and is equal to about a 0.1-per-cent. solution of glucose.

Under pathologic conditions the reducing power of urine may be enormously increased, due to the abnormal presence of glucose. This may be present up to as much as fifty grains to the ounce (10 per cent.).

The detection of sugar in the urine is based upon a knowledge of the following properties of glucose:

1. In hot alkaline solutions, reduces the oxides of copper and bismuth.

2. With brewer's yeast, ferments, forming alcohol and carbon dioxid.

3. Enters into chemical combination with phenylhydrazin to form characteristic insoluble crystals of glucosazone.

4. Deflects polarized light to the right.

In the application of all tests but the fermentation-test, it is necessary to use urine free from albumin. If this be present it must first be removed by the addition of dilute acetic acid and boiling to cause precipitation. This is then filtered out, and the filtrate used in the tests for sugar.

Reduction Tests.—Method of Fehling[3] (for preparation of reagent see Appendix): The sample of urine to be tested is first examined carefully for the presence of albumin. If this be

[3] The suggestions for the performance of this test are taken from a communication by C. W. Louis Hacker, M.D., appearing in the Jour. Amer. Med. Asso., page 252, Jan. 25, 1908.

found it must be removed by heat and acetic acid, and the filtrate used for the test. About 4 cubic centimeters of freshly prepared Fehling's solution are diluted with three parts of water, and brought just to the boiling point in a clean test-tube. Immediately two drops of proteid free urine are added, and the tube shaken vigorously. If the urine contains more than 2 per cent. of sugar, a yellowish-red precipitate immediately appears, changing rapidly to a brownish-red or bright red, which settles out slowly, leaving a greenish-blue supernatant fluid. If the reaction be positive, it is better to roughly dilute the urine with two or four parts of water, and to perform the test again.

With the strengths of dextrose from ½ to 2 per cent., two drops of urine, as stated above, will cause a characteristic precipitation of red suboxid of copper, about which there can be no doubt. With smaller percentages the change may come slowly, but even then heat need not be applied after the addition of the urine. Here little change may be seen in the Fehling's solution by transmitted light, but by reflected light a brownish-red tint is evident, which finally develops into a decided light-red precipitate, gradually settling out and leaving a clear, blue, supernatant fluid. The pentoses, lactose, and even maltose, by this method, do not give the characteristic reaction. Urines showing this reaction always give a reaction with Nylander's solution (see next page).

If no change occurs in the Fehling's solution after the addition of the two drops as above, the solution may then be warmed just to the boiling point, and from two to four drops of urine added and the mixture observed. This should be repeated until in all twenty drops of urine have been added. If no change occurs at this point, the urine does not contain dextrose to the extent of 0.5 per cent.

Cautions.—The disadvantages of using large amounts of urine (an equal or even one-half volume of the Fehling's solution) are two-fold. In the first place, the strongly alkaline copper solution with urine of high specific gravity throws down a more or less voluminous precipitate of cupric phosphate, which takes out the blue color of the solution, and changes it to a dirty, dark green. In the second place, the alkaline solution, acting on the ammonium salts in the urine, liberates sufficient ammonia to

hold in solution small amounts of the red suboxide of copper, obviously interfering with the detection of traces of dextrose. Very frequently under these conditions, and especially if the solution has been boiled, even for a few seconds after the addition of the urine, the greenish solution slowly takes on a dirty, yellow-brown color, then become turbid, and, eventually, on standing, there separates out a finely divided opalescent, greenish-yellow precipitate, which only settles out completely after the tube has been allowed to stand over night. In certain cases this reaction does not begin until a few seconds have elapsed after the addition of the urine. Such a reaction may be termed a pseudo-reaction.

Fallacies.—Neglect of the above precautions will result in the occurrence of the pseudo-reaction in about 5 per cent. of normal urines examined.

The following substances, if present in the urine in more than normal amounts, will result in partial reduction of Fehling's solution. Uric acid, creatinin, hippuric acid, mucin, hypoxanthin, and alkapton. Also the presence of the oxidation products of the following drugs: The alkaloids, arsenic, carbolic acid, and hexamethylamin (urotropin). The latter substance does not ordinarily reduce bismuth.

Bottger's Bismuth Test.—Urine containing coagulable proteids must first have them removed by heat and acetic acid. Mix in a clean test-tube equal volumes of urine and liquor potassii, and add a few grains of bismuth subnitrate and boil. In the presence of glucose the white powder in the bottom of the tube will gradually become gray, brown, or black.

Nylander's Bismuth Test.—Nylander modifies the preceding test by substituting for the subnitrate a special reagent containing bismuth (for reagent see Appendix). Albumin, if present in the urine, must be removed in the usual way. To 10 cubic centimeters of Nylander's reagent, add 1 cubic centimeter of urine and bring to the boiling point. A brown or black discoloration of the liquid denotes sugar.

Fallacies of bismuth tests. A slight reduction of bismuth may be caused by turpentine, eucalyptus, or the ingestion of large amounts of quinine. Albumin and sulphur compounds in urine produce a black precipitate.

Delicacy.—These tests show the presence of glucose in 0.025-per-cent. solution.

Trommer's Test.—To half inch of urine in a test-tube, add one inch of liquor potassii and a few drops of a 10-per-cent. solution of copper sulphate sufficient to cause blue discoloration with slight cloudiness. Heat this mixture to the first signs of boiling. If sugar be present the copper will be reduced to yellow- or red-oxid. Slight precipitations, which may occur after prolonged boiling, are no proof of sugar.

Delicacy.—This test, if carefully performed, will demonstrate the presence of sugar in 0.025 per cent. solution.

Modifications of Trommer's Test (Simrock).—Mix equal parts of the Simrock reagent (see Appendix) and urine in a test-tube and boil; the presence of glucose will be indicated by a yellowish or red discoloration and precipitate.

Phenylhydrazin Test.—This test depends upon the production of characteristic crystals of phenylglucosazone by combination of glucose and phenylhydrazin in hot solution, and their recognition with the aid of a microscope.

The Test.—Fill a beaker half full of water and warm upon the wire-gauze over a low Bunsen flame. Place a test-tube, containing the urine, which has been acidulated with a few drops of dilute acetic acid, in the beaker. While waiting for this to warm prepare the following solution (this must be made fresh, as it does not keep well): Weigh out one gram of phenylhydrazin hydrochlorid, and two grams of sodium acetate. Mix the salts and dissolve in 10 cubic centimeters of distilled water acidulated with two drops of dilute acetic acid. Filter to clarify. After the water in the beaker has boiled for five minutes, observe the urine in the tube; if it has become turbid, filter. To 10 cubic centimeters of hot urine in a clean test-tube, add 5 cubic centimeters of the filtered reagent, replace in a beaker of boiling water, and continue boiling for one hour. Then cool quickly by immersing the tube in cold water. If sugar was present in the urine a crystalline precipitate will appear. This should be taken up in a pipette and transferred to a microscope slide for examination. The characteristic crystals of phenylglucosazone are fine, faintly yellow needles arranged in the forms of fans, rosettes, and sheaves.

Fallacy.—Levulose, if present in the urine will produce similar crystals; these can be differentiated by a comparison of the fusing points of the two compounds.

Phenylglucosazone fuses at 204° C.

Phenyllevulosazone fuses at 150° C.

Delicacy.—Glucose forms characteristic crystals when present in 0.025 per cent. solution.

QUANTITATIVE ESTIMATION OF GLUCOSE.

Volumetric Determination by Fehling's.—Measure into a beaker or porcelain dish 10 cubic centimeters of accurately prepared Fehling's solution, and 40 cubic centimeters of distilled water. Heat the mixture over wire gauze to boiling. Prepare a dilute solution of suspected urine by adding nine parts of distilled water to one part of urine. This mixture is placed in a burette. Now to the boiling solution add the dilute urine, a few drops at a time, from the burette. Continue adding, boiling, and stirring until the blue color of the Fehling's solution completely disappears when viewed by transmitted light. Note accurately the number of cubic centimeters of urine used.

Fehling's solution is a standard solution of copper sulphate, 10 cubic centimeters of which is exactly decolorized by 0.05 gram of glucose.

EXAMPLE.—Suppose 8 cubic centimeters of diluted urine were used in decomposing 10 cubic centimeters of Fehling's solution. Then, of undiluted urine, 0.8 cubic centimeter was required. This amount then contained 0.05 gram (10 x 0.005) glucose. To calculate the percentage of sugar in the sample of urine: 0.8 : 0.05 :: 100 : X, which, in this case, "X" equals 6.25 per cent. To calculate the grams of glucose voided in twenty-four hours: If 1500 cubic centimeters of urine were voided in twenty-four hours, then 0.8 : 0.05 :: 1500 : X, which equals 93.75 grams glucose.

Caution.—The dilute urine should be added very slowly with frequent boilings and examinations by transmitted light to avoid passing the end reaction.

Robert's Differential Density Method.—This method depends for its result upon the alteration in density occurring from the fermentation of saccharine urine.

The Test.—Mix thoroughly about 120 cubic centimeters of urine with half a cake of compressed yeast. Take and record accurately the specific gravity of this mixture. Set aside in a warm place (thermostat preferred) for twenty-four hours, and at the expiration of this time again take the specific gravity and subtract the second reading from the first. Each degree of the remainder (showing density lost) represents approximately one grain of sugar to the ounce. To obtain the percentage of sugar in the specimen, multiply the degrees of density lost through fermentation by the factor 0.219.

Example.—Specific gravity before fermentation.....1041
Specific gravity after fermentation.....1023

Degrees of density lost..... 18

This is approximately the grains of glucose in each ounce of urine. 18 x 0.219 equals 3.94% glucose.

This test is easily performed, and is conclusive evidence of the presence of glucose. It is, however, not strictly accurate, and is not to be relied upon when the amount of glucose present is less than 0.5 per cent.

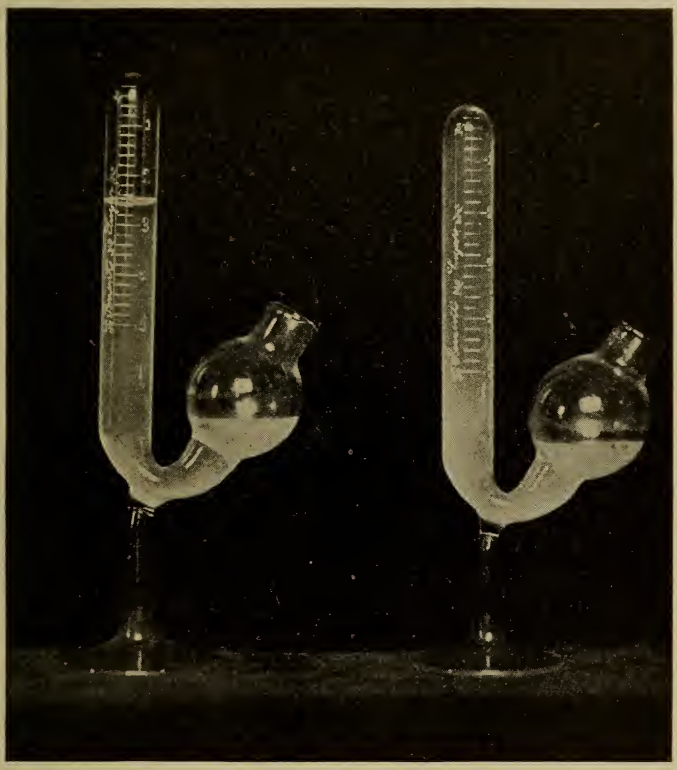
FERMENTATION SACCHARIMETER.

None of the substances found in the urine, which give the reduction reactions resembling glucose, are susceptible of alcoholic fermentation. This fact establishes the accuracy of the test. The test itself depends upon the fact that when sugar-containing urine is mixed with a quantity of brewer's yeast and kept in a warm place for twenty-four hours, it will ferment, giving off bubbles of CO_2, and at the same time forming alcohol.

By measuring the amount of carbon dioxid gas, formed during this fermentation-process, we are enabled to estimate the percentage and amount of sugar contained in the specimen under examination. A specially shaped and graduated tube (see Fig. 25) is usually employed in this test. It is known as the Einhorn saccharimeter. The upright tube is graduated from its closed upper end downward from 0.1 to 1.0 per cent. It will not estimate sugar in amounts less than 0.1 per cent., and owing to the varying solubility of the various gases of the urine, depend-

ing on differing reactions and the density, it cannot be regarded as absolutely accurate.

The Test.—Urines containing less than 5 per cent. of glucose must be diluted five times with water. Urines containing more than 5 per cent. must be diluted ten times before proceeding with the test.



A B

Fig. 25.—Einhorn Saccharimeter During Performance of Test. A, Showing 2½ Per Cent. Sugar. B, Control with Normal Urine.

Mix in a large test-tube about 20 cubic centimeters of properly diluted urine with one-twelfth of a cake of fresh, compressed yeast. Completely fill the upright tube of the saccharimeter with this mixture, so as to exclude all air from the graduated tube. Fill a second saccharimeter with yeast dissolved in distilled water or with urine known to be free from

sugar. These tubes are to be kept in a warm place and allowed to remain undisturbed for from eighteen to twenty-four hours. By this time the sugar-containing urine will be found to have been displaced by gas in the vertical tube. The percentage corresponding to the level of the fluid will represent the percentage of sugar in the diluted urine. This figure multiplied by the dilution, will represent the percentage of sugar in the specimen under examination.

Cautions.—The urine must be faintly acid, and must be so diluted that the specific gravity will be less than 1008, and when diluted must contain less than 1 per cent. of glucose. If any gas is liberated by the yeast or fluid in the control-test, this amount must be subtracted from that indicated in the test-mixture before computing the percentage.

The internal administration of mercuric chlorid, iodoform, salicylic acid, hexamethylamin, quinine, and other antiseptic drugs will inhibit fermentation, and therefore must be excluded before testing.

PURDY'S QUANTITATIVE METHOD.

Into a beaker or boiling flask of 250 cubic centimeters capacity put 35 cubic centimeters of Purdy's reagent (see Appendix) and 70 cubic centimeters of distilled water. Boil steadily over a wire gauze, and add urine slowly from a burette until the blue color begins to fade; now proceed cautiously, and after the addition of each drop wait for a few seconds to see if the end reaction is complete. Continue adding until the solution of the reagent is colorless and transparent. To obtain this result the total amount of urine employed must have contained 0.02 gram of glucose.

Example.—Suppose the amount of urine employed to completely decolorize the reagent was 4.5 cubic centimeters, then 4.5 cubic centimeters of urine contained 0.02 gram of glucose; or 1 cubic centimeter of urine contains 0.0044 gram of glucose. This multiplied by 100 will give the percentage of glucose which is equal to 0.44. If the color of the reagent is changed by less than 4 cubic centimeters of urine, it is best to dilute the urine with one or two parts of water, and then multiply by this factor to obtain the final result.

This method is rapid, the technic is simple, and the end-reaction definite and sharp. The reagent is stable.

CAMMIDGE REACTION.[4]

This reaction is based upon the presence of certain, at present unknown, substances, which occur in the urine in pancreatic disease associated with fat necrosis, the detection of which is based upon some special reactions in which phenylhydrazin hydrochlorid plays an active part.

A-Reaction.—The specimen of urine to be examined is filtered, and 10 cubic centimeters of the filtrate are placed in a small flask; 1 cubic centimeter of hydrochloric acid (specific gravity 1.16) is added, and a funnel placed in the neck of the flask to act as a condenser. The flask is then set up on a sand-bath and gently boiled for eight minutes; then, after rapid cooling in running water, the contents are filtered.

To 5 cubic centimeters of this filtrate add 5 cubic centimeters of distilled water. The excess of acid is now neutralized by the slow addition of four grams of lead carbonate; then, after standing for a few minutes to allow completion of the reaction, the urine is filtered through a wet filter and the flask washed out with 5 cubic centimeters of distilled water through the filter. To the clear filtrate now add two grams of pulverized sodium acetate, and 0.75 gram phenylhydrazin hydrochlorid. This mixture is now boiled for from three to four minutes on the sand-bath. The hot fluid is then immediately poured into a test-tube and allowed to cool undisturbed. After the lapse of a variable period, depending on the severity of the case, of from one to twenty-four hours, a more or less flocculent precipitate or yellowish deposit accumulates in the bottom of the tube. This precipitate, when examined under the microscope, is found to consist of sheaves and rosettes of golden yellow crystals.

As a necessary preliminary to this test, sugar, if any be present in the specimen, must be removed by fermentation, and the alcohol formed driven off by heat. Albumin should be removed by the addition of dilute acetic acid, heating and filtering.

[4] See "Diseases of Pancreas," Robson and Cammidge, 1907, for further information.

When the A-Reaction is positive then the B-Reaction must be made.

B-Reaction.—Twenty cubic centimeters of urine are filtered and mixed with 10 cubic centimeters of a saturated bichlorid of mercury solution. After filtering, the filtrate is allowed to stand for a few minutes, and then to 10 cubic centimeters of this filtered mixture add 1 cubic centimeter of concentrated hydrochloric acid. This is then placed in a small flask and transferred to a sand-bath, and allowed to boil for ten minutes. Subsequently to 5 cubic centimeters of this mixture add 10 cubic centimeters of distilled water. After cooling, this is neturalized with four grams of lead carbonate. The remaining stages are the same as in the A-Reaction.

The B-Reaction is a differential one based upon the observation of Cammidge, that in inflammatory conditions of the pancreas the crystals formed in the A-Reaction are destroyed by the mercuric bichloride.

A large number of observations showed Cammidge that the crystals in malignant disease are broad and coarse, while in inflammatory processes they are smooth and slender.

The solubility of the crystals in 33 per cent. sulphuric acid also differs according to the nature of the affection.

The crystals obtained from a case of acute pancreatitis are soluble in from one-quarter to one-half minute; in chronic pancreatitis in from one-half to two minutes; in carcinoma in from three to five minutes or longer.

Negative results with these reactions have been obtained by Cammidge,[5] and also by Robson,[6] in normal urines, in jaundice, in gall-stones without pancreatitis, in gastric ulcer, and in a number of other conditions.

In support of the practical value of these reactions it may be stated that Felix Eichler[7] has lately produced acute pancreatitis in dogs, and in the urine of each he found the characteristic crystals, while the reaction applied to normal control dogs it was consistently negative.

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[5] Cammidge: Lancet, March 19, 1904.
[6] Mayo Robson: Laucet, page 773, 1904.
[7] F. Eichler: Berlin. klin, Wochenschrift.

POLARIMETRIC METHOD.

Because of its ability to turn the ray of polarized light to the right, glucose is called dextrose. The accurate determination of glucose in urine may be made by those possessing polariscopes. The degree of dextro-rotation can be read on a graduated scale and calculated as percentage or grams of glucose.

Since albumin in solution deflects the polarized ray to the right, this must first be removed by acidulating, boiling, and filtering. To make an accurate estimation the urine must be first decolorized by shaking with a piece of lead acetate and filtering.

Delicacy.—Instruments vary in delicacy. Usually distinct dextro-rotation may be detected when glucose is present in 0.025 per cent.

Fallacy.—Maltose, which rarely occurs in the urine, if present, will deflect the ray even more than will glucose.

(For description of technic see larger works on clinical chemistry or polarimetry.)

CLINICAL SIGNIFICANCE OF GLYCOSURIA.

The presence of glucose in the urine is pathologic. If the amount be abundant and persists, associated with copious water-drinking and eating, while the patient is at the same time emaciating, it is indicative of diabetes mellitus. The urine in this condition is usually pale with a fruity odor, and while overabundant, has a high specific gravity. As much as 10,000 cubic centimeters of urine may be excreted in twenty-four hours, at the same time the specific gravity may be maintained at 1050.

Temporary Glycosuria.—The appearance of small amounts of sugar may occur transiently in the urine after excessive ingestion of sugar; and reducing substances, such as glycuronic acid, may give a spurious positive reaction. Copper reduction by such substances is not corroborated by either fermentation or the phenylhydrazin tests.

Glycosuria also may accompany certain diseases of the brain, spinal cord, and pancreas, or be a transitory accompaniment of phthisis, pneumonia, cirrhosis, or cholera.

LACTOSEURIA.

Milk sugar occurs occasionally in the urine of nursing women, particularly toward the end of lactation. It will reduce Fehling's and Nylander's solutions, but returns a negative result with the phenylhydrazin test. It ferments very slowly.

MALTOSEURIA.

Maltose has been found in the urine of diabetic patients, and when present reduces copper and ferments with yeast. Its occurrence is sufficiently rare to be discounted in the clinical laboratory.

PENTOSEURIA.

Diabetics and morphine habitues occasionally show in the urine traces of pentoses. These will reduce copper, but will not ferment.

Newmann's Orcin Test.—This test may be employed to distinguish between the pentoses, the glycuronates, and dextrose.

TECHNIC.—Three cubic centimeters of urine are treated with ten drops of a 5-per-cent. alcoholic orcin solution and 10 cubic centimeters of glacial acetic acid. The mixture is now brought to the boiling point and allowed to cool. After cooling concentrated sulphuric acid is added, drop by drop, and shaken until about twenty drops have been added. In the presence of pentoses the color becomes olive-green, glycuronates turn the solution violet, and dextrose gives a carmine color.

ACETONE.

Usually in advanced diabetics, sometimes in fever or in perfect health, particularly in patients whose diet is rich in proteids, the urine may have an ethereal odor, and will give positive reactions to tests for acetone.

In the course of a case of diabetes a decline in the percentage of sugar, accompanied by lowered specific gravity, but without corresponding improvement in the general symptoms, may indicate impending coma. At this time acetone will be found in the urine in increasing amounts, with or without diacetic acid and oxybutyric acid.

Ethylene-Diamin-Hydrate Test.—This is the simplest, and is probably the most satisfactory for clinical purposes.

TEST.—Place in a clean test-tube about 5 cubic centimeters of distilled water, and to this add about one grain of sodium nitro-prusside; shake to effect solution. Add to this an equal amount of suspected urine and thoroughly mix. Gently overlie the mixture with a few drops of a 10-per-cent. solution of ethylene-diamin-hydrate. A pink or ruby-red color at the zone of contact denotes the presence of acetone. A faint white cloud, which appears on the addition of the reagent, does not denote acetone. The solution of sodium nitro-prusside must be freshly prepared.

LEGAL'S TEST.—To 5 cubic centimeters of urine in a test-tube, add a few drops of a freshly prepared solution of sodium nitro-prusside and a few drops of sodium hydrate solution. A red color will appear in all urine, which soon changes to yellow. If this is now overlaid with strong acetic acid a change in color at the line of contact from yellow to carmine or purplish-red indicates acetone.

DIACETIC ACID.

Coincident with the appearance of acetone in diabetic urine, we may have diacetic acid.

TEST.—To 5 cubic centimeters of urine in a test-tube, add two drops of ferric chlorid solution; if a precipitate appears, filter and add more ferric chlorid to the filtrate. The presence of diacetic acid will be indicated by a portwine discoloration.

Fallacy.—Antipyrine, salicylic acid, and coal-tar derivatives will give a similar reaction if they have recently been ingested by the patient.

OXYBUTYRIC ACID.

This substance cannot be readily detected by chemical means unless a long and difficult preliminary process is resorted to in order to separate it from the other urinary constituents. These methods will be found in larger works on clinical and physiologic chemistry.

BILE PIGMENTS.

General Considerations.—Bile pigments are never found in the urine under normal circumstances. As a rule the freshly voided urine only contains the oxidized derivative, bilirubin. If a cystitis should exist, then subsequent oxidation products, biliverdin, bilifuscin, biliprasin, and bilihumin may also be encountered.

Bile-containing urines present a more or less characteristic appearance; their color may vary from a bright golden-yellow to a dark greenish-brown. On microscopic examination it is usual to find the morphologic elements of the urine stained yellow or reddish-brown. The same color may be imparted to the foam on shaking.

Gmellin's Test for Bile-Pigments.—Put some yellow nitric acid in a test-tube and gently overlay it with the suspected urine. In the presence of bile-pigment a play of colors will be observed at the zone of contact. Green will be seen nearest the urine and orange in the upper part of the nitric acid. This test is exceedingly sensitive, indicating the presence of bile-pigment in a dilution of 1 to 80,000.

Nitric Acid Paper Test.—Moisten a piece of white filter or blotting paper with the suspected urine, and place it on a glazed tile or slab of porcelain. Allow one or two drops of commercial nitric acid to fall into the center of the wet paper. In the presence of bile-pigment concentric rings of blue, violet, green, and yellow will appear. A slight red reaction cannot be considered positive, as it may be produced by other substances than bile-pigment.

Import.—When bile is not freely and normally discharged from the bile-passages, the coloring matter from the retained bile is absorbed by the lymphatics, the various body tissues become stained with it, and it is partly eliminated in the urine.

BILE ACIDS.

The presence of bile-acids in the urine has the same clinical significance as the presence of biliary pigment. The tests for the acids are fraught with considerable difficulty, and do not compensate in their significance for the time and energy expended.

It will, therefore, usually be found sufficient to determine the presence of bile-pigments.

UROBILIN.

General Considerations.—Urobilin is closely allied to the normal urochrome, but is an abnormal product, probably resulting from the action of reducing agents upon the normal urinary pigment. It is said to be identical with the stercobilin of the feces, and its behavior to chemical reagents can be tested by testing an alcoholic extract of feces.

Test for Urobilin.—(a) Mix some urine in a test-tube with an equal amount of a 10-per-cent. solution of zinc acetate. Filter off the precipitate, and the filtrate, in the presence of a demonstrable quantity of urobilin, will show a beautiful greenish fluorescence. With the polariscope the urobilin in this solution gives a distinct absorption spectrum.

(b) Fill a clean test-tube three quarters full of urine, and add one drop of strong hydrochloric acid; to this add about one-sixth volume of amylic alcohol, and after shaking slowly eight or ten times allow to separate by standing. Pour off the supernatant fluid and add to it thrice its volume of alcohol. Prepare separately a 5-per-cent. solution of zinc chlorid, and add one drop of this to the alcoholic extract of the urine; finally add one drop of ammonium hydroxid. Zinc hydrate will be precipitated, which should be filtered off. In the presence of urobilin the filtrate will present a beautiful green fluorescence.

Significance.—Urobilin has been detected in cases of hepatic cirrhosis, malarial anemia, carcinoma, Addison's disease, and pancreatic disease with acholic stools.

HEMATURIA.

General Considerations. — Blood may gain access to the urinary tract in many ways, and appear in the urine in varying amounts, from the smallest trace, demonstrable only by chemical means, to sufficient to produce bloody urine with clots.

Blood from the kidney is usually well mixed with the urine, to which it imparts a brownish smoky hue. Under the microscope tube-casts of blood-cells may be found.

Blood from the ureter may be well-mixed with the urine, or may appear in characteristic worm-like clots.

Blood from the bladder may, or may not, show irregular clots. When recently shed it imparts a bright red color to the urine; it is frequently accompanied by much mucus and large, flat epithelial cells in great numbers.

Blood from the prostate, examined by the three-glass test, appears in the first and third glasses only.

Blood from the urethra appears in the first glass, and is frequently clotted.

Urine may show the presence of blood in the absence of any demonstrable lesion of the genito-urinary tract. It has been noted after the ingestion of strawberries, gooseberries, or a large amount of rhubarb.

The blood may be contaminated from menstrual discharge. This possibility should always be borne in mind, and false conclusions guarded against.

Microscopic Appearance.—The most convenient method of demonstrating blood in the urine is by microscopic examination of the centrifugated or sedimentated sample. The corpuscles in ordinary acid urine maintain their characteristic bi-concave shape for a number of days, if decomposition and putrefaction are prevented. If the urine is of high specific gravity, the cells rapidly become crenated. On the other hand, if the urine be alkaline or becomes so after voiding, the corpuscles will appear swollen, shriveled, or shadowy. Urine containing more than a trace of blood is albuminous.

Test for Occult Blood in the Urine.—To 10 cubic centimeters of urine in a large test-tube, add about twice as much ether and agitate thoroughly by pouring from one test-tube to another several times. To this add a few grains of powdered gum guaiac, and again agitate. Next add 5 cubic centimeters of glacial acetic acid (99.4 per cent.) and again agitate; allow this to settle and then pour off the supernatant liquid and divide equally between two test-tubes; set one aside for a control, and to the other add about 2 cubic centimeters of fresh hydrogen dioxid from a pipette, making an effort to have it settle to the bottom as a distinct layer. If a bluish discolora-

tion appears either at the zone of contact or throughout the mixture, the presence of blood is demonstrated.

HEMOGLOBINURIA.

This term signifies the presence of hemoglobin in the urine free from blood-corpuscles. Besides the foregoing test it may be demonstrated by means of the spectroscope. Faintly-acid urine containing traces of hemoglobin will give two characteristic absorption bands of oxyhemoglobin. By the addition of a minute quantity of ammonium sulphid, the spectrum is changed to that of reduced hemoglobin.

Hemoglobinuria occurs in a variety of conditions: scurvy, pyemia, purpura, typhus fever, poisoning from arsenic, phosphorus, carbolic acid, chloral, and potassium chlorate. There has also been noted a periodic form of obscure origin.

PYURIA.

Pus being an albuminous fluid, will cause urine containing more than a minute quantity to respond to the tests for albumin. Urine containing much pus is turbid, and deposits on standing a white or greenish-white sediment which is insoluble in heat and in dilute mineral acids. The addition of hydrogen dioxid produces rapid effervescence.

Microscopic examination of pus-containing urine will reveal the characteristic pus corpuscles. These are spheric, granular, and highly refractile. The nucleus is usually obscured or disintegrated.

Donne's Test for Purulent Sediment.—To the suspected sediment add a small piece of caustic potash, and stir with a glass rod. Pus will become thick, tough, and gelatinous, while mucus will become flaky and thin.

Significance of Pyuria.—The presence of pus in the urine indicates the presence of an inflammatory process in the genito-urinary tract, the location of which can, in some measure, be determined by the character of the associated epithelial cells (see page 218).

EPITHELIA

The epithelial cells found in urine may come from any part of the genito-urinary tract. Their forms vary greatly, and with a knowledge of the characteristic cells belonging to the different regions, it is possible, with more or less certainty, to determine their origin by their appearance. The puss cell may be taken as the standard of size.

Cells from the tubules of the kidney are round and about one-third larger than a pus cell.

From the pelvis of the kidney, twice the size of a pus cell and cuboidal or pear-shaped.

From the ureter, round and slightly smaller than from the pelvis.

From the bladder, flat and square. These are the largest cells encountered, except those from the vagina.

From the urethra, smaller than from the bladder; they may be cuboidal or columnar. All epithelial cells are granular and contain a relatively small nucleus.

TUBE CASTS.

General Considerations.—In the presence of albuminuria or hematuria, microscopic examination of the urinary sediment will, as a rule, reveal the presence of tube casts. Occasionally some varieties of casts will be found in urine which show neither albumin or blood.

The Sediment.—In order to obtain a sediment for microscopic examination, some method of precipitating and of concentrating this precipitate is necessary for a conclusive examination. A centrifuge (Figs. 26, 27 and 28) is the most rapid as well as the safest method to employ. This process only requires from 15 to 20 cubic centimeters of urine, and can be accomplished in a few minutes. After centrifugation a part of the supernatant urine is poured off and then, with the aid of a pipette with a small point, a drop or two is drawn up and transferred to a clean microscope slide for examination.

The method of employing a conical glass may be used if a centrifuge is not at hand. Sedimentation by this means requires a number of hours, and, if care is not taken to prevent

bacterial contamination and if the specimen is not kept in a cool place, the specimen may decompose and the morphologic elements be destroyed.

The sedimentation glass is valuable for roughly estimating the amount of gross sedimentation in phosphaturia, pyuria, etc. (Fig. 29.)

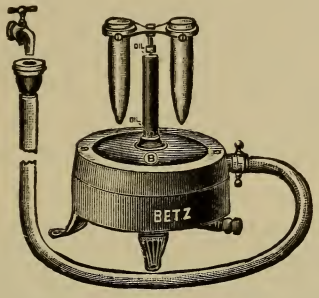


FIG. 26.—WATER-POWER CENTRIFUGE.

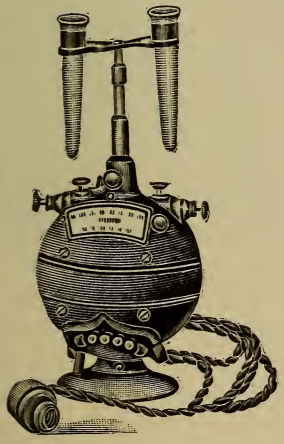


FIG. 27.—ELECTRIC-POWER CENTRIFUGE.

FIG. 28.—HAND-POWER CENTRIFUGE.

Varieties of Casts.—HYALINE CASTS are almost transparent and appear as ground glass. They have a delicate but definite outline, and are quite friable.

Strong illumination of the field may obscure them entirely, as they are very delicate in outline and structure. They are best seen with the plane side of the reflector, and side illumination which should not be too bright.

The particular significance of hyaline casts is not yet posi-

tively settled. They occur in advanced grades of nephritis and again in transient albuminurias, and even in the absence of demonstrable albumin; they are frequently seen during the course of fevers, particularly typhoid fever.

Granular Casts.—These are more opaque than the hyaline, and are, therefore, more easily seen. The granules are numerous, and upon close examination will be found to permeate the matrix of which the cast is partly composed. This will serve to differentiate them from the pseudo-granular casts, which are merely hyaline casts to which granular *débris* has become attached during centrifugation or sedimentation. These latter

Fig. 29.—Sedimentation Glasses.

A Clear. B, Slightly cloudy with beginning precipitation. C, Sedimentation complete.

are of no more significance than hyaline casts. Granular casts are probably composed of a hyaline matrix which has undergone degeneration. They frequently show fragmented cells and fat globules in their structure. The continued occurrence of hyaline and granular casts in the presence of a permanent albuminuria, usually denotes chronic interstitial nephritis.

Hyalo-granular Casts. — Hyaline casts are occasionally found, parts of which are distinctly granular throughout their substance. They apparently represent a stage of cast-formation between the hyaline and granular varieties, and their significance is probably the same as hyaline casts.

Epithelial Casts. — These casts are composed of renal

epithelial cells (slightly larger than pus cells), grouped in the form of a short cylinder and cemented together with a hyaline or mucoid matrix. The cells may be either whole or fragmented, clear, opaque, or granular. They are significant of an acute desquamative process, resulting from renal inflammation.

Fatty Casts.—These casts may possess any of the characteristics of the preceding varieties, and present in addition free fat-globules scattered throughout the cast. They are considered as proof of fatty degeneration of the kidney.

Blood Casts.—These casts are composed either of coagulated blood, in which innumerable corpuscles in various stages of disintegration are embedded, or they may represent a hyaline matrix in which appears a varying number of red blood-cells. Some hyaline casts, which show a few blood-cells adherent upon their surfaces, may be simply hyaline or granular, to which the red cells have become attached in the bladder or after voiding. The presence of true blood casts indicates hematuria of renal origin.

Bacterial Casts.—These resemble the granular variety, except that they are more closely and more uniformly granular. The bacteria may be so numerous that the cast is almost opaque. They denote the occurrence of an acute bacterial infection of the kidney, and are rarely found.

Waxy Casts.—These are of rare occurrence, and are probably simply a dense variety of the hyaline cast.

Crystalline Casts.—As the name implies, they are crystalline in nature, being composed usually of uric acid, and more rarely of oxalates. They are very rarely encountered.

CYLINDROIDS.

These are narrow, ribbon-like bands which usually present longitudinal striations, which may or may not extend throughout the entire length of the cylindroid.

They are essentially hyaline in nature, and are probably formed from a hyaline basis. They are of renal origin, and are encountered in about 75 per cent. of cases which ordinarily come to the physician in the course of practice.

Their significance is slight, though they are considered to

indicate a degree of kidney irritation. In this connection it is of interest to note, that when found, they are many times accompanied by a high specific gravity, and oxaluria, with or without the presence of indican.

Microscopic Appearance. — Cylindroids appear as faint, ribbon-like bands, seen best by low illumination, and frequently exceeding in length the diameter of the field of the microscope when viewed through an objective of moderate power. Longitudinal striations may usually be detected, often running through only a portion of their length, the ends of which, after making one or more graceful curves, terminate in a gradual taper.

Cylindruria with Albuminuria.—This condition may occur as a transitory phenomenon after such violent exercises as bicycle racing, foot-ball or rowing; also in chronic constipation or after the ingestion of moderate or large amounts of alcohol, particularly by those unused to it. Continued ingestion of the salicylates has been found to produce it. It is often present during attacks of intestinal indigestion associated with oxaluria.

SPERMATURIA.

The presence of semen in the urine will give positive reactions for albumin. It may be present in the urine after coitus, after nocturnal emissions, or in spermatorrhea.

Microscopic examination will reveal the characteristic elements which are motionless, but which resist decomposition for days.

CHYLURIA.

Under very occasional circumstances the urine may contain chyle, which gives it the appearance of milk, and forms on standing a cream-like layer at the top. It responds to all the tests for albumin.

Microscopically, much fat is found in the form of innumerable highly refractive globules, which are soluble in ether.

THE INORGANIC SEDIMENT.

To obtain a specimen of inorganic sediment, it is preferable to centrifuge the freshly voided urine rather than to wait for the slower process of sedimentation. The use of the centrifuge precludes the possibility of the process of decomposition so altering the specimen that the original picture is destroyed.

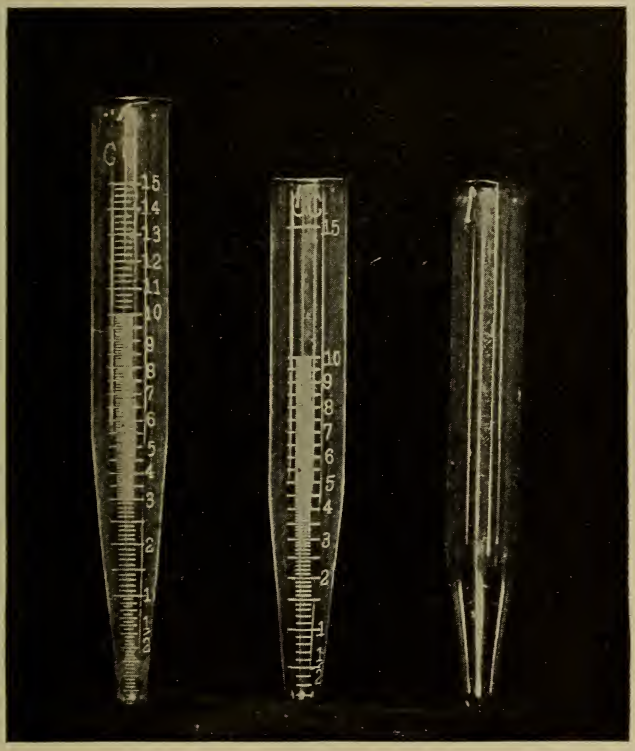


FIG. 30.—CENTRIFUGE TUBES.

A, Correct form of graduated percentage tube (note the slender taper which facilitates reading of small per cent.). B, Improper form of graduated tube accurate reading of small percentages difficult. C, Plain centrifuge tube.

Alteration in reaction will so change the character of the crystalline deposit that it will be useless from a diagnostic standpoint. A decomposed alkaline urine will often present such a mass of phosphatic sediment that the less voluminous, but more important, elements are greatly obscured and may be overlooked.

Preparation of the Slide.—The sediment should be taken from the bottom of the centrifuge tube (Fig. 30) by means of a small pipette with a long, slender tip. Not more than a drop or two should be allowed to enter the tube. This is particularly important when the amount of sediment is small. If the sediment is large and dense it should be diluted with a drop or two of distilled water and a cover-glass placed upon it. Much time will be saved by first examining the preparation with the ⅓ or ¼ objective, which is convenient for locating an interesting part of the slide, upon which the high-power objective may be focused for more careful study.

Crystalline Deposits. (See Plates IV, V and VI.)

Acid Group.—*Uric Acid* (Plates IV and VI): These crystals are yellow, reddish-brown or brown in color. The most characteristic forms are rhombic prisms or lozenge-shaped crystals (Plate IV, Figs. *a* and *b*). These occur singly, but more often they are united in irregular masses. (Other more rare forms are shown in Plate IV, Figs. *c*, *d*, *e* and *f*; Plate VI, *a* and *b*.)

Urates.—The urates, chiefly the urate of sodium and the urate of potassium, if they do not appear as an amorphous deposit, appear as crystals in the forms of needles or dumb-bells, of reddish-brown color, and also in globular masses which are dark-brown and almost opaque, with or without projecting spines.

Oxalates.—The usual form of calcium oxalate in the urine is a perfect octahedron without color. More rarely they appear in the conventional hour-glass form (Plate V, *a* and *b*). This form is somewhat similar to the urate from which it may be distinguished by the total absence of color in the oxalates.

Carbonates.—These are rare, but when present evolve bubbles of gas when treated with hydrochloric acid under the microscope.

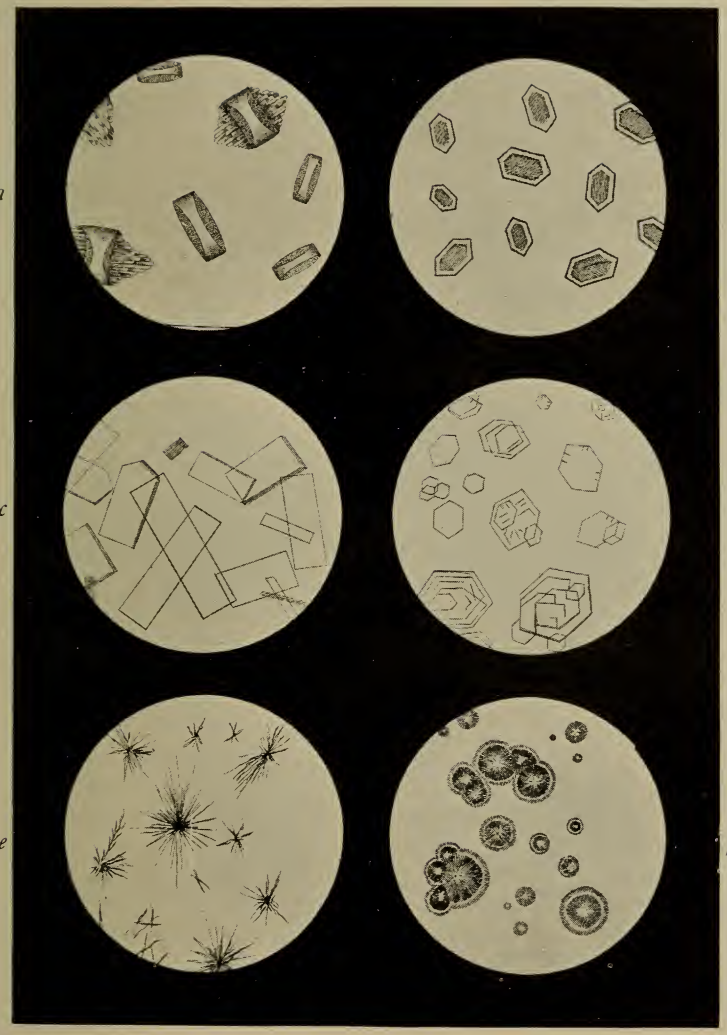
Sulphates.—This is a rare form of deposit which, when present, appears as fine, feathery crystals. Frequently a number of crystals radiate from a common center.

Alkaline Group.—*Phosphates:* These may occur as a semi-opaque amorphous deposit without color. More commonly they appear as the characteristic coffin-lid crystals. A less com-

PLATE IV.

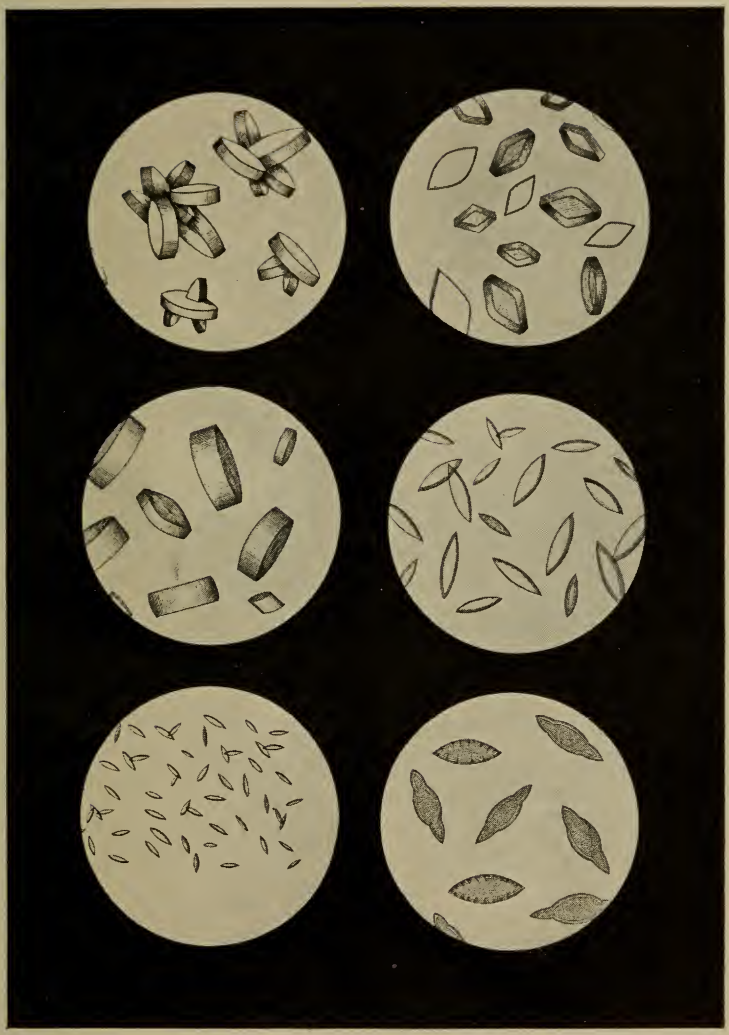
a and *b*. Calcium Oxalate Crystals. *c*, *d*, *e* and *f*. Phosphates.

PLATE V.



a and *b*. Uncommon Forms of Uric Acid Crystals. *c*. Cholesterin. *d*. Cystin. *e*. Tyrosin. *f*. Leucin.

PLATE VI.



a and *b*. Usual Forms of Uric Acid Crystals. *c*, *d*, *e* and *f*. Less Common Uric Acid Crystals.

mon form of crystalline phosphatic deposit (Plate V, *c*, *d*, *e* and *f*) appears as fine, branching, feathery, crystals, which have been likened to the needles and branches of the pine tree.

Ammonium Urate (see Fig. 23).—These are characteristic of the uric acid and urate group in that they are yellow or brownish in color. In alkaline urine the urates appear as fine, feathery spheres of varying size, resembling to some extent chestnut burrs.

Cholesterin.—This is a rare form of deposit which appears in the form of irregular, flat platelets whose sides follow the characteristic lines of a parallelogram, the angles of which are often irregular. Not infrequently the platelets occur in overlapping groups. (Plate VI, *c.*)

Cystin.—This is a rare deposit. When present it appears as irregular transparent plates of varying size often in overlapping groups. (Plate VI, *d.*)

METHOD TO DETERMINE ROUGHLY THE NATURE OF THE COMMON UNORGANIZED URINARY DEPOSIT.

1. Warm the deposit and some urine. If it dissolves, the deposit is composed of urates. If it is unaffected by heat, the deposit is either phosphates, uric acid, or calcium oxalate.

2. Warm a fresh portion of urine with acetic acid. If the sediment dissolves, it is composed of phosphates; if it does not dissolve, it is uric acid or calcium oxalate.

3. Add to this undissolved portion some HCl and heat again. If the sediment now dissolves, it is calcium oxalate; if it does not dissolve, it is composed of uric acid.

URINARY CONCRETIONS.

General Considerations.—About 75 per cent. of all urinary concretions are either uric acid or urates. Next in frequency are found the calcium oxalate or mulberry concretions. More rare primary concretions may consist of blood, cystin, xanthin, calcium phosphate, or calcium carbonate.

Secondarily, any one of these formations may become covered with a whitish layer of mixed phosphates. These are pre-

cipitated upon the original concretion by ammoniacal fermentation, which occurs in the bladder, secondary to the presence of the calculus.

Analysis of the Concretion.—1. Burn a portion upon a piece of platinum foil in a Bunsen flame or blow-pipe.

A. If it chars greatly and leaves little ash, it may be uric acid, urates, cystin, xanthin or blood.

1. If it gives the murexid test, it is uric acid or urates.
2. If it dissolves in boiling water, it is urates.
3. If it does not dissolve in boiling water, it is uric acid.
4. Cystin and xanthin are very rare forms of concretions which require special tests for their detection.

B. If it chars very slightly and leaves considerable ash it may be phosphates, oxalates or calcium carbonate.

1. Treat a fresh portion with dilute HCl.
   (a) If it dissolves with effervescence, carbonates are present.
   (b) If it is soluble without effervescence. phosphates or oxalates are present. (See C, 3, below.)
2. Treat a portion with dilute acetic acid.
   (a) If it dissolves with the aid of heat, it is phosphatic.

C.—1. If it fuses to a bead on platinum foil, it is urates.

2. If it does not fuse, it is calcium phosphate.

3. If it is insoluble in either of the above acids, it is calcium oxalate.

THE DIAZO REACTION.

The Reaction.—The ammoniacal solution of the anhydride of para-diazo-benzin-sulphonic acid has the property of developing a salmon pink or red color reaction with certain pathologic urines. Normal urine never gives this reaction.

The Test (for preparations of solutions see Appendix).—Take 5 cubic centimeters of solution "A" and add three drops of solution "B," and mix together in a clean test-tube. To this

add 5 cubic centimeters of urine and again mix. Now allow a few drops of ammonia water to flow down the side of the tube. As it comes in contact with the mixture in the tube a pinkish or red color indicates a positive reaction. Upon shaking the tube the entire mixture becomes colored, and the color is also imparted to the foam. This reaction does not always appear immediately, so a few minutes should be allowed for its development before reporting a negative reaction.

Significance of the Reaction.—It is not yet definitely known what particular substance or substances in the urine yield this reaction. It may occur in the presence of several aldehydes, ketones, and phenols.

A positive reaction is usually obtained with the urine of typhoid fever patients after the fourth day of the disease. Its continued absence, however, does not preclude the possibility of this disease. It is also positive in some cases of measles, pulmonary tuberculosis, etc.

EXAMINATION OF THE URINE FOR SUBSTANCES INTRODUCED INTO THE BODY FROM WITHOUT (Drugs and Poisons).

Detection of Lead.—A bright strip of magnesium, free from lead, is placed in the urine and left there for some time; the deposit which forms is dissolved in nitric acid, and then tested according to the methods of inorganic analysis.[7]

If the urine contains but a small amount of lead this method will not give the desired result; then a larger quantity of urine must be used, the organic substances destroyed with HCl and potassium chlorate, and the lead sought for in the evaporated residue.

Detection of Mercury.—To 500 to 1000 cubic centimeters of urine, add 2 to 4 cubic centimeters of HCl; then digest at 60° to 80° C., for five or ten minutes in a flask with a few bright strips of brass or copper. The metal is then washed with water, then with alcohol, and finally with ether, and dropped into an ignition tube (glass tube of high-fusing point). This is then brought to a red heat, care being exercised to keep the

[7] See Marshall's Medicus, J. B Lippincott Co.

upper end of the tube cool. The mercury which has become amalgamated with the metal becomes volatilized, and is redeposited in the upper cold end of the tube, where it is seen as a bright mirror, or, if a small amount, the individual globules of mercury may be seen by a hand-lens or the low power of the microscope.

Detection of Iodine.—Iodine occurs in the urine as potassium iodide after the internal or external application of iodine or one of its combinations. It may easily be demonstrated as follows: A few cubic centimeters of urine are boiled with a piece of starch of about the size of a pea until the latter is dissolved. After cooling, the fluid is carefully overlaid with concentrated nitric acid. If iodine is present a blue-violet ring, which gradually disappears, is formed at the line of junction of the two fluids. A second method is to add to the urine a few drops of crude nitric acid and a less number of drops of chloroform, and then shake gently. If iodine is present the chloroform, which sinks to the bottom of the tube, will be colored rose-red or violet. Both the above tests are very delicate, but if the urine contains only a small trace of iodine, the chloroform-test is not very conclusive, since the nitric acid may set free indol and skatol pigments, as well as uroresein, any of which will color the chloroform in a similar manner. But in this case the urine itself appears more deeply colored than the chloroform.

Detection of Bromine.—The test for bromine is performed in exactly the same manner as iodine-test, except that a few drops of a calcium chlorid solution and hydrochloric acid are used to liberate the bromine. If bromine is present the chloroform will be colored yellowish-brown. This test, though far less delicate than the iodine-test, is sufficiently accurate to recognize the therapeutic ingestion of large doses of bromine salts, and is chiefly of interest in verifying the diagnosis of suspected bromism.

This method may be uncertain because of the discoloration of the chloroform by urinary derivatives. To prevent this source of error 16 cubic centimeters of urine, to which have been added 2 cubic centimeters of caustic potash and 2 cubic centimeters of potassium nitrate, are evaporated and incinerated, the ash

dissolved in water, and the resulting solution tested for the presence of bromine, as above.

Detection of Salicylic Acid.—Dilute ferric chlorid is added to the urine drop by drop. If the latter becomes a more or less intense violet, the reaction is positive. Salicylic acid and its salts, in which latter form the administered salicylic acid partly appears in the urine, both give this reaction.

Detection of Phenol.—Phenol appears in the urine largely as phenol sulphate. Ferric chlorid will produce a violet-blue color in the distillate from phenol urine, to which 5 per cent. sulphuric acid has been added. Phenol urine turns dark or black on exposure to the air, when sufficient time is allowed for oxidation to occur.

Detection of Antipyrin.—The urine appears dark and is dichroic, *i.e.*, in reflected light, greenish; by transmitted light, reddish. A permanent brown-red color gradually appears upon the addition of ferric chlorid solution.

Detection of Phenacetin (acetphenetedin).—The urine is dark yellow and turns reddish-brown on the addition of ferric chlorid solution. The color gradually becomes black after prolonged standing.

Detection of Antifebrin.—The urine is extracted with chloroform, and to the extract mercuric nitrate is added. The mixture is then heated, and if a green color is produced antifebrin is present.[8]

Detection of Pyramidon.—The urine is frequently clear, purplish-red in color, and deposits a sediment composed of small, red needles. If the urine is mixed with an equal volume of a 2-per-cent. solution of ferric chlorid, a dark-brown, amethyst color is produced.

Detection of Tannin.—Tannin is eliminated in the urine partly as gallic acid. Urine which contains tannic and gallic acids turns a deep blue-black upon the addition of ferric chlorid solution.

Detection of Balsam of Copaiba and Sandalwood Oil.—After the administration of copaiba the urine will reduce cupric oxid (Trommer's test), but not bismuth (Nylander's test). If HCl

[8] Yvon, Jour. de Pharm. et de Chemie, No. 1, 1887.

is added to the urine drop by drop, a precipitate of resinous acids appears with a reddish or violet coloration. Also after the administration of sandalwood oil the urine has reducing properties, and exhibits a precipitate of resinous acid when HCl is added, but it will be of a reddish-brown color.

Detection of Santonin.—Santonin urine is of a saffron-yellow or greenish color. The addition of sodium hydrate will turn it a rose-red. If this rose pigment is shaken with amylic alcohol, it will be immediately taken up by the latter, giving it an intense and beautiful color, while at the same time the urine is decolorized.

XII.

THE CEREBROSPINAL FLUID.

To Obtain the Specimen.[1]—It is very important when withdrawing the cerebrospinal fluid to see that too much is not abstracted at one time. Death has resulted from too rapid and too great removal of fluid. This risk need hardly be considered in obtaining fluid for purposes of examination, as not more than 8 to 10 cubic centimeters are required. Untoward symptoms are less likely to follow spinal puncture if the patient is kept in bed for a few hours succeeding the procedure.

The control of the needle is important. It is quite possible with an 8-centimeter needle to thrust into the peritoneal cavity or between the bodies of the vertebra, and by this latter path to pierce even the vena cava. Aspiration to start the flow is not permissible, as with proper precautions and careful technic a dry tap is very rare.

The Pressure.—For exact pressure-determinations elaborate apparatus is not required. For ordinary bedside work the apparatus about to be described is sufficient: As soon as the subarachnoid space has been entered, as shown by the appearance of fluid in the needle, a thick-walled glass tube of fine bore (about 1½ millimeter diameter) is joined to the needle by a short rubber connection, and the fluid allowed to find its level in this tube held vertically, and the pressure measured in terms of millimeter according to the height to which the fluid rises. The capillary error, variations due the differing specific gravity in the fluid, and the change due to loss of fluid from the spinal canal, may be neglected for all practical purposes. Under ordinary bedside conditions, with this apparatus, the normal variations in cerebrospinal pressure are between 50 and 300 milli-

[1] Abstract of article by Francis Peyton Rous, M.D., in International Clinics, Vol ii, Seventeenth Series.

meters of the fluid itself. By careful experimentation and elaborate apparatus, the normal variations have been found to lie between 120 and 180 millimeters of distilled water.

Physiologic Modifications.—Crying or coughing during the examination, will cause a rise in pressure of 50 millimeters or more. Posture also influences pressure, as is shown if the patient (who should be reclining horizontally on one side) raises his head. The column further shows fluctuations synchronous with the pulse and respiration. Finally the absolute pressure will be higher than normal if the patient exerts any muscular force during the examination. Muscular exertion should be eliminated as much as possible during the reading.

Pathologic Modifications.—Marked increase in pressure is the rule in hydrocephalus, in brain tumor, and in meningitis of bacterial origin, especially when the infection is acute. Increase in pressure often accompanies uremic coma. A column of 600 to 700 millimeters of fluid is not uncommon in any of these conditions. Low pressure is observed in infants in conditions giving low-blood pressure (see section on "Blood-pressure"), and when an error in technic has occurred.

Collection of the Specimen.—When the pressure has been determined, disconnect the manometer and collect a few cubic centimeters in a sterile capillary tube as the fluid emerges from the needle. This specimen should be preserved under aseptic precautions for bacteriologic examination; from 4 to 6 cubic centimeters more should be allowed to run directly into a clean centrifuge tube for cytologic examination.

Determination of the Cell-Content.—In the normal cerebrospinal fluid there are from one to seven white cells per cubic millimeter. Probably they are never entirely absent. When the meninges are sound none but lymphocytes occur; these are often much degenerated. The fluid is normally clear, and even if it contains several hundred cells per cubic millimeter, it may remain clear macroscopically.

The cells may be counted with the Thoma-Zeiss hemocytometer, using the "red" pipette for measuring. Since there is usually some hemorrhage accompanying the puncture, it is necessary to have previously counted the number of red and white cells in the patient's blood to determine the ratio of these

cells in the circulation, so that after counting the red and white cells in the cerebro-spinal fluid it will be possible to determine how many white cells are adventitious (due to hemorrhage). To determine the cell-content of the cerebrospinal fluid it is only necessary to place a drop of the freshly drawn fluid upon the depression in the chamber and apply the cover-glass; to count the red cells one filling will be all that is necessary. For the white cells, if the cells are scant, five or ten complete fields must be counted and an average obtained.

EXAMPLE.—Suppose in the specimen we find 80,000 red cells and 102 white cells per cubic millimeter, while in the blood itself there are 4,000,000 reds and 5000 whites, or a proportion of 800 to 1. Calculating on this ratio of the 102 white cells present per cubic millimeter of the cerebrospinal fluid, 100 are from the contamination blood. Thus, but two white cells per cubic millimeter were present in the fluid before contamination by hemorrhage.

Pathologic Variations in the White Cells.—The cell-content of the cerebrospinal fluid is an extremely sensitive index of the state of the meninges.

ACUTE MENINGITIS.—Purulent fluids may contain from 4000 to 40,000 white cells per cubic millimeter.

TUBERCULAR MENINGITIS will average between 200 and 300 cells per cubic millimeter, although it may be as high as 20,000.

SYPHILITIC MENINGITIS, tabes, paresis, usually give more than 10 and less than 100 cells per cubic millimeter.

Caution.—It must be borne in mind that the occurrence of a slight increase in the number of white cells may be the result of a previous puncture. This question should be ascertained before arriving at final conclusions in any case.

It must be remembered, further, that in cerebrospinal fluid kept at room-temperature for a short time, the cells rapidly degenerate so that they are often unrecognizable after a lapse of a few hours, even if they have not entirely disappeared.

Differential Count.—In general, should the pressure of the fluid be between normal limits, and the cells less than 10 per cubic millimeter, it is unnecessary to make a differential count. Under these circumstances lymphocytes alone are present. The differential count should be accomplished speedily after

removal of the fluid to prevent alteration due to decomposition. The fluid which has been allowed to fall into the centrifuge should be revolved rapidly for from ten to thirty minutes. This completed, the tube should be carefully removed from its metal sheath to avoid dissipating the cells. To transfer the sediment to a slide or cover-glass the fluid must be drained away by slowly and steadily inverting the tube. While the tube is still inverted the slight portion of fluid remaining in the end of the tube is taken up on a capillary pipette and blown out on to the previously cleaned glass. This is air-dried, when it is ready for fixing or staining (for methods of staining for differential count see section on "The Blood").

Lymphocytes alone (and perhaps occasionally a large, flat endothelial cell) are normal to the fluid. In the differential count from the stained specimen, it is necessary to count and classify the polymorphonuclears, the mononuclears, and endothelial cells. Mast cells, eosinophiles, tumor or nerve cells, have rarely been detected, and must still be considered exceptional, if not doubtful. The relationship of the polymorphonuclears and the lymphocytes are alone of importance in our present state of knowledge.

Polymorphonuclears are indicative of an acute process, while the mononuclears speak for chronicity. The mononuclears (lymphocytes of blood) appear to be particularly numerous in tubercular meningitis and cerebrospinal syphilis.

Determination of Proteid Content. — The fluid remaining after the bacterial examination, and that supernatant after centrifugation, may be devoted to the proteid determination. An albumin and a globulin have been found in the cerebrospinal fluid, the relative importance of each has not yet been determined, so for clinical purposes their combined content alone is determined. The acetic-acid boiling-test may be applied here, provided the examiner is familiar with the normal amount of proteid as represented by the white cloud formed in the upper half of the tube. For a rough quantitative determination a very narrow test-tube may be marked off in lengths to correspond to those of the Esbach tube. The Esbach reagent is used as in the similar test for albumin in urine. This test will serve to show the proteid variations from time to time in a given case.

There is an increase in the proteid content of the cerebrospinal fluid in all inflammations of the meninges. Proteid increase and cell increase usually go hand in hand, but this relationship is not constant.

Interpretation of Findings.—Having ascertained the pressure, the cell-content and cell-character, and the amount of proteid in any given case, one is in a position to state much.

1st. As to the presence or absence of meningeal infection, if the fluid be clear and colorless macroscopically, the cells less than 8 per cubic millimeter, and the proteid content low with the pressure between 50 and 300 millimeters of the fluid itself, this, as far as is at present known, may be considered conclusive evidence against an active pathologic change in the meninges.

2d. A red-yellow or brown fluid under increased pressure (above 300 millimeters) showing erythrocytes, and increased proteid content, is evidence pointing to hemorrhage into the subarachnoid space.

3d. When tabes, paresis or cerebrospinal syphilis is suspected, a specimen showing moderate cell increase (10 to 200 cells per cubic millimeter), mononuclear in character, with or without proteid increase, and the pressure not above 300, speaks for a chronic process.

4th. When meningitis is in question a definite increase in cells (over 100), specially of the polymorphonuclear variety, increase in proteid, and pressure greater than 300 millimeters, is strongly in favor of a diagnosis of acute meningitis, even in the absence of macroscopic change in the fluid and a negative bacteriologic report.

5th. Cases of brain tumor and of hydrocephalus usually show an increase in pressure, with a normal cell-content.

Bacteriologic.—For staining method see section on "Bacteriologic Methods."

XIII.

THE BODY FLUIDS.

THE PERITONEAL FLUID.

Characteristics of Normal Fluid.—In conditions of health there is just sufficient fluid to thoroughly lubricate the interior of the abdominal cavity. This fluid is clear, of a pale, straw color, having a specific gravity of 1005 to 1015; is slightly albuminous and, under the microscope, a drop of freshly-drawn fluid placed between a clean slide and cover-glass, reveals very few, if any, formed elements.

Pathologic Exudate. — INFLAMMATORY ASCITIC fluid is straw or lemon-yellow in color, and usually somewhat cloudy, depending upon the number of cells contained in it. Its specific gravity varies between 1012 and 1026, or even higher. The fluid frequently coagulates spontaneously. It shows a variable amount of albumin by boiling, and sugar, bile-pigments, urea, uric acid, and cholesterin may be demonstrated by appropriate means (see section on "Urine"). More rarely xanthin, creatinin, and allantoin may be found.

HEMORRHAGIC EFFUSION occurs in cancer and tuberculosis of the abdominal cavity, and occasionally in cirrhosis of the liver.

A CHYLOUS or MILKY FLUID is occasionally met. This is readily distinguished by examination of fresh fluid under the microscope, when the characteristic fat globules will be found.

Non-Inflammatory Transudate. — In Bright's disease the ascitic fluid is usually a pale clear serum of low specific gravity, showing a minimum of cells.

In cirrhosis of the liver the color is usually darker and bile-pigment can be demonstrated.

Differentiation. — OVARIAN CYSTS: The fluid obtained from ovarian cysts has a specific gravity usually above 1030, and

is of a dark-brown, grumous appearance. Ovarian fluids are further said to contain a large number of compound granule cells which, from their frequent occurrence in this fluid, have been termed "ovarian cells." Microscopically these appear as large oval or round cells, showing dense, irregular granulations often obscuring the nucleus of the cell.

PLEURAL FLUID.

General Considerations.—Physiologically there is not present enough fluid for analysis. Under pathologic conditions it may vary from one to four or more liters, and may be serous, sero-purulent, purulent or hemorrhagic.

Non-Inflammatory Transudate.—In hydrothorax the specific gravity, as a rule, is below 1015, the albumin content is small, and there is little tendency for the fluid to coagulate spontaneously. The fluid is clear or pale, straw-colored, unless tinged with hemorrhage occurring during the puncture. The formed elements are very scarce and hard to demonstrate.

Inflammatory Exudates.—Sero-Fibrinous Pleurisy: The serous exudate is abundant, and flakes of fibrin are present in the fluid, appearing as fibrillated flocculi. The actual amount of fluid varies greatly. It is of a citron color, either clear or slightly turbid from the fibrin and formed elements present. In some cases the color is dark-brown, usually due to altered blood contained in it. Specific gravity below 1020.

Microscopically, we find a variable number of leucocytes and red blood-cells, and some swollen endothelial cells and bacteria.

Purulent Pleurisy.—The specific gravity is usually above 1020. The fluid has a heavy, sweetish odor, which, if the infection be due to a penetrating wound, may be fetid. The fluid is essentially pus, contains a very large amount of albumin which may coagulate spontaneously after removal. Appropriate tests may show cholesterin, uric acid, bile-pigment, and sugar.

Method of Making Permanent Stained Preparation.—In fluids which are clear and show but few cells on microscopic examination of the fresh specimen, it becomes necessary to centrifuge 15 to 20 centimeters in order to concentrate these formed

elements. After centrifugation in the ordinary urine centrifuge for from two to three minutes, the supernatant liquid is slowly and steadily poured off, and the remaining residue taken up in a capillary pipette from which it is blown out upon clean cover-slip and allowed to dry spontaneously (without heating). It then may be stained by any of the Romanowski stains (see section on "Blood") or the eosin-methylene-blue sequence.

Fluids which contain cells in macroscopic amount may be transfered to cover-glasses for staining without centrifugation.

Microscopic Examination of Stained Preparations. — In hydrothorax the cells are few and mainly large, flat, of endothelial origin.

In pneumo- and strepto-coccic pleurisy, there is a great preponderance of polymorphonuclears. In tubercular inflammations the lymphocyte is the predominating cell.

Many efforts at classification and diagnosis have been made on a limited number of examinations with a view to arriving at some definite rules for cytodiagnosis of pleural effusions. Further systematic study along these lines is, however, necessary.

The presence of red cells is usual in tuberculous processes, also in the presence of rupture of an abscess from an adjacent organ into the pleural cavity. Very frequently red blood-cells, in a pleural effusion, are due to the traumatism inflicted at the time of puncture. Red cells which appear in the first few centimeters drawn, are most likely of this origin.

The possibility of diagnosing cancer by microscopic examination of the fluid is always present, through the finding of characteristic cell masses in the fluid.

THE PERICARDIAL FLUID.

This fluid normally is of a pale, lemon-yellow color, slightly viscid, cloudy from cell detritis; occasionally it may be clear. It contains a moderate amount of albumin and certain inorganic salts. The specific gravity ranges between 1015 and 1030.

THE SYNOVIAL FLUID.

This fluid is alkaline, thick, viscid, sticky, and of a yellowish color. Physiologically the joints contain just sufficient to

completely lubricate them. Under pathologic conditions the fluid, in a large joint, may amount to many cubic centimeters.

HYDROCELE FLUID.

The fluid is usually clear, of a yellow or greenish tinge. The specific gravity varies between 1014 and 1026. The fluid sometimes coagulates spontaneously. Some leukocytes are always present, and occasionally crystals of cholesterin.

XIV.

HUMAN MILK.

General Considerations.—It must always be remembered, in the examination of milk, that it is no simple matter to obtain a truly representative sample. The fatty matters tend to separate, and there may be great variations between different portions of the same sample. This difficulty is particularly pronounced when human milk is examined, because of changes in the milk incident to mental and nervous disturbance.

The Sample.—The sample should consist of a thorough mixture of portions taken at separate intervals, by means of the breast-pump. Wherever possible it is well to have the woman somewhat accustomed to the use of the pump before taking any milk for examination. In all cases the sample should be thoroughly mixed before testing, by pouring rapidly from vessel to vessel.

Physical Characteristics.—Woman's milk is bluish-white in color, of sweetish taste and characteristic odor. When freshly drawn it is alkaline or amphoteric, but never under healthy conditions is it acid. The specific gravity varies between 1026 and 1036, the average being 1031 at 60° F. On the addition of acetic acid only a slight coagulum is seen, being in the form of small, fine flocculi, never in large masses as is the case with cows' milk. Besides the myriad fat-globules, may be seen large, flat epithelial cells from the milk ducts.

Composition of Human Milk (after Holt).

	Average.	Normal variations.
Fat	4.00 per cent.	3.00 to 5.00 per cent.
Sugar	7.00 " "	6.00 to 7.00 " "
Proteids	1.50 " "	1.00 to 2.25 " "
Salts	0.20 " "	0.18 to 0.25 " "
Water	87.30 " "	89.82 to 85.50 " "
Total	100.00 " "	100.00 to 100.00 " "

The composition and food value of human milk are impaired when the mother is unhealthy. It is affected injuriously when there exists undue emotional excitement. It will contain an appreciable amount of certain medicines ingested by the mother, which may affect the infant.

Examination of the Milk.—The exact determination of the composition of human milk is only to be determined by a complete chemical analysis. There are, however, many variations from the normal which the physician may readily ascertain for himself by simple methods of examination.

The Quantity.—This may be determined roughly by using the breast-pump, although this is not reliable for many reasons. With sufficiently sensitive scales the physician may, by weighing the infant before and immediately after nursing, determine whether it is getting only one or two, or four or five ounces. The average daily quantity secreted by woman is one liter or two pints.

The Reaction.—Test by litmus paper. The reaction should be *alkaline* or *amphoteric*, but never acid when freshly drawn.

A spontaneous change occurs after milk has stood for some time in a warm place; it coagulates or sours, and becomes acid. This is due to the change of milk-sugar (lactose) into lactic acid. This occurs through the agency of the *bacterium lactis*. The casein normally is held in solution by the alkaline phosphates, the acid changes the reaction, and hence causes the casein to be precipitated.

Specific Gravity.—This may be taken by a small hydrometer which is graduated from 1010 to 1040. The specific gravity is lowered by fat, but increased by the other solids.

Microscopically, milk is found to be composed of minute brilliant, oil globules encased in a thin envelope of casein. Immediately after delivery the milk is relatively poor in casein, but rich in fatty matter, which exists in considerable amount in the form of colostrum masses. The microscope also reveals the presence of colostrum corpuscles, blood, pus, epithelium, and granular masses. Colostrum corpuscles are abnormal after the twelfth day; blood and pus are always abnormal.

Determination of the Fat.—The simplest method is by

means of the cream gauge. This consists of a graduated test-tube with a foot, and a ground-glass neck and stopper. The tube is filled to the zero mark with freshly-drawn milk and allowed to stand at room-temperature for twenty-four hours, when the percentage of cream is read off directly from the graduated scale. The relation of cream to fat is approximately five to three. Thus 5 per cent. cream equals 3 per cent. fat, etc.

Centrifuge Method.—The use of a specially graduated centrifuge tube is more accurate. These tubes may be used in the ordinary centrifuge for urine. Only 6 cubic centimeters of milk are required for this test. If carefully conducted the test is nearly as accurate as the chemical analysis. It gives results accurate to within one-fifth of 1 per cent.

In the usual apparatus two pipettes are supplied with the centrifuge tubes, one of 5 cubic centimeters capacity, marked milk, the other holding 1 cubic centimeter up to a mark, for introducing the alcoholic solution.

To determine the fat by this method, 5 cubic centimeters of the sample is introduced into the tube by means of the pipette marked "milk," 1 cubic centimeter of the alcoholic solution (solution-A) is added, and the tube well shaken. Then, by means of any large pipette, solution-B is added little by little until the tube is filled to the zero mark. It is then placed in the centrifuge and rotated very rapidly for four or five minutes. This will bring the fat to the top in a clear yellowish layer which can be read off in direct percentage by the scale on the neck of the tube.

A few drops of water may be added, if necessary, to correct the level of the top of the fluid. If the milk should be richer than 5 per cent. for it will be necessary to dilute the sample by an equal quantity of water, proceed with the test as above, and finally multiply the result by two.

Solution-A consists of:—

Amylic alcohol	37 parts by volume.
Methyl alcohol	13 parts by volume.
Hydrochloric acid	50 parts by volume.

This solution may be kept for a short while; if it turns dark it is worthless. Solution-B consists of sulphuric acid, specific gravity 1832.

Determination of the Sugar.—The percentage of sugar is nearly constant, so it may be ignored in the usual clinical investigation.

Estimation of the Proteids.—There is no simple method of determining the percentage of proteids. If we regard the sugar and salts as constant, or so nearly so as not to effect the specific gravity, we may form an approximate idea of the proteids from a knowledge of the specific gravity and the percentage of fat. We may thus determine whether they are greatly in excess or very scanty. The specific gravity then will vary with the proportion of proteids, directly, and inversely with the proportion of fat, *i.e.*, high proteids, high specific gravity, low fat, low specific gravity. The application of this principle will be seen by reference to the accompanying table.[1]

Composition of Woman's Milk.

Specific gravity at 70° F.	Cream 24 hrs.	Proteids Calculated.
Average 1031	7 %	1.5%.
Normal variation 1028-1029	8% to 12%	Normal (rich milk).
Normal variation 1032	5 to 6%	Normal (fair milk).
Abnormal variation. Low (below 1028)	High (above 10%)	Normal or slightly below.
Abnormal variation. Low (below 1028)	Low (below 5%)	Very low, very poor milk.
Abnormal variation. High (above 1032)	High	Very high, very rich milk.
Abnormal variation. High (above 1032)	Low	Normal or nearly so.

[1] From Holt's "Pediatrics."

XV.

BACTERIOLOGIC METHODS.

By a gradual process of evolution and development the field of clinical medicine has so enlarged its borders that to-day the laboratory worker no longer confines his investigations to examination of the blood, urine, and sputum, etc., but must possess a working knowledge of bacteriology and be familiar with bacteriologic technic to the extent that he may be able to obtain, differentiate, and recognize the commoner pathogenic organisms. This section will confine itself to a brief outline and discussion of the essentials of laboratory technic, referring the worker, who would delve further into this fascinating field, to works devoted to bacteriology.

The Tubercle Bacillus.—This is the most important organism, from a clinical standpoint, at least, which is encountered in the field of clinical medicine.

The tubercle bacillus presents great difficulties in the way of artificial cultivation, and the results of such procedures are so unreliable that they are rarely depended upon for purposes of identification, the diagnosis usually being made upon the greater acid-fast properties of this organism as compared with all others; the final corroboration of the diagnosis being made by animal inoculation, for which purpose the guinea-pig, owing to its great and uniform susceptibility, is commonly employed.

The tubercle bacillus is, in the strict sense of the word, a parasite which finds conditions entirely favorable to its growth and reproduction only in the body. On artificial media the bacilli, even when transferred directly from the human body, grows only imperfectly or not at all.

For artificial cultivation of the tubercle bacillus, the best results are obtained by the use of some blood-serum medium (see page 267).

The finding of tubercle bacilli in the sputum is positive

evidence of pulmonary, bronchial or laryngeal tuberculosis. On the contrary, their absence, after careful search, even of a number of preparations, cannot be considered absolute negative evidence.

If, after careful search of suspicious particles taken from the sputum of a suspect, whose clinical history and symptoms are strongly suggestive of the disease, the organism is not found, the following devices may be resorted to:—

A. The whole amount of a twenty-four hours' specimen of sputum, to which no antiseptic solution has been added, should be placed in a porcelain dish or glass beaker, and stirred with a glass rod until quite thin and diffluent. This should then be stood aside for a few hours to settle, when the lowest portion of the fluid is taken up in a pipette and transferred to a centrifuge-tube, where it is rotated for from fifteen minutes to half an hour. From the bottom of the centrifuge-tube a small amount of the sediment is transferred to a cover-glass or slide for fixing and staining.

B. A considerable quantity of sputum is mixed with an equal quantity of water, and a few drops of a 10-per-cent. solution of sodium hydrate is added. This mixture is then heated until homogeneous, when it is cooled, sedimented, and centrifuged, and examined as outlined above.

Microscopic Appearance.—The tubercle bacillus is a delicate rod usually appearing in stained specimens with a beaded internal structure. It may be straight, but is usually slightly curved in its long axis. Its length is very variable, some being short, others quite long, though never as long threads. Its average length varies between 2 and 5 micro-millimeters, and it is usually very slender.

In sputum the organisms may occur singly or in groups of from three to half a dozen or more.

Staining Peculiarities.—The recognition of the tubercle bacillus depends upon a special method by which they alone are stained. Unstained they cannot be differentiated from the other organisms which may be present. The ordinary methods employed for staining bacteria are not suitable, so that special technic has been devised and is now regularly employed to render their recognition less difficult.

In these methods advantage is taken of the fact that certain substances increase the activity of staining by aniline dyes. With the tubercle bacillus this is accomplished with carbolic acid. Another important point is that these organisms, when once stained, give up their color only with great difficulty, so that agents, which will decolorize all other bacteria in the course of a few minutes, will have no appreciable effect upon the tubercle bacillus. It is upon these two peculiarities that we rely in differentiating this organism.

Differential Diagnosis.—While the peculiar micro-chemical reaction toward staining reagents is unually considered to be unique with the tubercle bacillus, it should be remembered that at least three other species of bacteria, when similarly treated, react in the same way. This fact is particularly important in connection with the microscopic examination of urine and pathologic secretions from the genito-urinary tract, and from the rectum. Here is commonly encountered the *smegma bacillus* which is the next most important member of the group of acid-fast organisms. Acid-fast bacilli have been found in the sputum and about the teeth and tonsils in a case of non-tubercular disease of the lung.[1]

While these other organisms have the same acid-fast property as has the tubercle bacillus, they appear so rarely that serious mistakes are not likely to occur. The method of Pappenheim (see page 247) may be relied upon to overcome this difficulty. This method colors the tubercle bacillus red and the smegma bacillus blue.

Staining Methods.—Ziehl-Nielsen: Place a few drops of the carbol-fuchsin solution (for preparation of stain see Appendix) upon the fixed cover-glass preparation, and hold over a low Bunsen flame until steam begins to rise. Do not boil. Continue the steaming process for from three to five minutes, pour off excess of stain, and wash in water. It is important to prevent the staining reagent from reaching the under surface of the cover-glass. If this is permitted the pigment will become dry and burned into the glass, when it will successfully resist all efforts at complete decolorization. Decolorize with acid-alcohol

[1] Pappenheim: Berlin. klin. Woch., No. 37, p. 809, 1898.

solution (see Appendix) or with 25 per cent. sulphuric acid. Decolorization should be continued until the red color has entirely disappeared from the specimen; if this process is not thoroughly done the finished slide, so far as its diagnostic value is concerned, will be worthless. The specimen is then washed in water, and counterstained for from one to three minutes with a 1-per-cent. aqueous solution of methylene-blue, after which it is washed, dried, and mounted for microscopic examination. By this method the tubercle will appear as the characteristic red rods upon a blue background. A field which reveals other red objects has been insufficiently decolorized and should be discarded for a more carefully prepared specimen.

Gabbett's.—Flood the dried and fixed specimen with carbol-fuchsin and steam for three minutes; then pass directly to the acid methylene-blue stain (see Appendix) for one minute. Finally wash, blot, dry in the air, and mount. This field, if properly prepared, should have the same appearance as that prepared by the Ziehl-Nielsen method.

Pappenheim recommends the following technic: 1. Stain in carbol-fuchsin by steaming near the boiling point for three or four minutes. 2. Pour off the excess of carbol-fuchsin and treat without washing with Pappenheim's solution (see Appendix), pouring it slowly three or four times over the preparation, and allow it to drain off. 3. Wash in water, dry, and mount. Duration of entire procedure from three to five minutes.

Czaplewsky's Method.[2]—This method employs the following solutions: *(a)* One gram of fuchsin is dissolved in 5 cubic centimeters of liquid carbolic acid in a dish; 50 cubic centimeters of glycerin are then added with constant stirring, and finally dilute with water to 100 cubic centimeters. The solution is said to keep extremely well, and does not need to be filtered. *(b)* Ebner's decolorizing solution (see Appendix).

Method.—1. Stain, with the aid of heat, for three or four minutes. 2. Decolorize by treating the specimen alternately with Ebner's fluid and distilled water. 3. Counter-stain with methylene-blue for one minute. 4. Wash, dry and mount in Canada balsam. The chief advantage of this method is said to

[2] Hyg. Rundschau, No. 21, 1896.

be that all acid-resisting bacilli but the tubercle bacillus, are decolorized, and therefore cannot be confused with the bacillus in question.

DIPLOCOCCUS PNEUMONII.

The presence of Frankel's diplococcus in the sputum of patients suffering with croupous pneumonia, is fairly constant, so that its demonstration is of considerable diagnostic importance. They appear as elongated lanceolate cocci, usually arranged in pairs with their bases approximated. They are surrounded with a faintly staining capsule which, in dry preparations, does not usually take the stain at all, although the ordinary method employing methylene-blue has occasionally, in the author's experience, demonstrated a faint but distinct capsule.

The organism is supposed to be the cause of lobar pneumonia, but must not be confounded with the other diplococci occurring in the sputum, more especially with Friedlander's bacillus. The latter also possesses a capsule, but has nothing whatever to do with the production of lobar pneumonia, though occasionally they may accidentally be present. Friedlander's organism, when highly magnified, will be found to be a short rod. Cultural characteristics will also serve to differentiate, as will also Gram's staining method, which decolorizes Friedlander's and stains Frankel's organism.

The following modification of GRAM'S METHOD will be found satisfactory (for preparation of staining reagents see Appendix): Hold the fixed and dried cover-glass preparation in the forceps and flood with carbol-gentian violet; allow this stain to act for from three to four minutes. Wash and transfer to the iodine-potassium-iodide solution for from one to two minutes. Next wash in alcohol until the apparently dirtily-stained film is decolorized. Transfer to absolute alcohol, then to oil of cloves, and finally mount in balsam. By this method the organism appears as a dark-blue or violet diplococcus. Friedlander's organism will remain unstained.

A very useful method for differentiating Frankel's coccus is that devised by W. Wolf. By this method the dry preparation is first stained in aniline water saturated with fuchsin, and then placed for one or two minutes in a dilute watery solution

of methylene-blue. The cocci will now be found stained blue, the capsule rose-red, and the body of the specimen purplish-red.

Method for Staining Capsules.—Prepare the cover-glass smear in the usual way, then without drying flood with glacial acetic acid. At the expiration of one minute pour off the excess of acid, and without washing flood the specimen with aniline water gentian-violet (Koch-Ehrlich), which should be allowed to act for four minutes, when the excess of stain is poured off and a fresh portion added, which is allowed to act for another two minutes. The cover-glass is now washed in two or three changes of normal saline solution (it may be found necessary to employ a saline solution of 1.5 to 2.0 per cent.), after which it is blotted, dried, and mounted in the usual way.

By this method, the capsule will appear as a faintly tinted halo about the diplococcus. The absence of this cocci practically excludes the diagnosis of lobar pneumonia, although its demonstrated presence is by no means positive evidence in the other direction, because this organism has repeatedly been demonstrated in the sputum and mouth secretions of healthy individuals.

BACILLUS OF INFLUENZA OR PFEIFFER'S BACILLUS.

A small slender bacillus occurring usually in very great numbers in the nasal secretion of fresh attacks of true influenza, but not found in the ordinary short attacks of prostration accompanied by coryza, which is at present designated influenza or "grippe," largely for lack of a more definite diagnosis. *Morphologically*, it is a very small rod appearing frequently in pairs; in parts of the secretion this organism may be found in what is practically a pure culture, occurring both within and without the leukocytes. Their length is usually from two to three times their width, they rarely form chains. The ends of the rods are rounded off, and if imperfectly stained (they stain with difficulty) the ends will be more deeply stained than the center, giving an appearance not unlike a diplococcus with just a suggestion of a capsule. According to recent authority this bacillus does not possess a capsule, the deceptive appearance being probably a staining peculiarity of certain cells. The bacillus is non-motile, and can only be cultivated upon special media contain-

ing hemoglobin. This characteristic will readily serve to differentiate it from the colon bacillus and other organisms of similar appearance. An easily made and satisfactory medium is prepared by spreading a little fresh blood upon the surface of an ordinary agar slant and inoculating this with the infected material. This organism only develops on artificial media between the temperatures of 26° and 43° C., growing best at body-temperature. Upon the blood-agar slant incubated at 37° C., there will develop minute, transparent, watery colonies that are without structure, somewhat resembling droplets of due. They are usually discrete, and show little or no tendency to coalesce.

Staining.—One of the best methods of staining is with a dilute watery solution of Ziehl's carbol-fuchsin (the color of the solution should be pale red). This solution should be allowed to act for five minutes. This organism is decolorized by Gram's method.

A second method of staining is with Loeffler's methylene-blue (for preparation see Appendix). This stain should be allowed to act for five minutes. Then wash in water, mount, and examine for blue organisms.

BACILLUS OF DIPHTHERIA.

From the grayish white deposit on the fauces of a diphtheritic patient, prepare a series of cultures in the following way:—

Have prepared a few tubes of Loeffler's blood-serum. Pass a stout platinum needle which has previously been sterilized into the membrane and rub it around there; then being careful that it touches nothing else, rub it carefully over the surface of two tubes. The tubes are then immediately replugged and placed in the incubator. If the case be one of true diphtheria, the tubes will be ready for examination the following day.

The blood-serum mixture is to be preferred to the ordinary plate method because the organism of diphtheria grows better on this medium than upon any other: it is also a differential method in a general sense, because other organisms do not grow well on Loeffler's serum, hence a luxuriant growth at the expiration of twenty-four hours should always be considered diphtheritic until proven otherwise.

Appearance.—After twenty-four hours the tubes present a characteristic appearance. Their surfaces are marked by more or less irregular patches of white or cream-colored growths, which is usually more dense at the center than at the periphery.

Staining.—From this culture smears are made upon clean cover-slips or slides, dried and fixed in the usual way, and then stained with Loeffler's alkaline methylene-blue. There will now be seen, in a typical case, upon microscopic examination, slightly curved bacilli of irregular size and outline. In some cases they will be more or less clubbed at one or both ends; sometimes they are spindle shaped or may present curved edges. They are rarely or never regular in outline. Many of these irregular rods are seen to be marked at circumscribed points in which their protoplasm is deeply stained. This irregularity in outline and appearance is the morphologic characteristic of the bacillus of diphtheria.

THE GONOCOCCUS OF NEISSER.

On **microscopic examination** the pus from an acute case of gonorrhea will show numerous small bodies, usually arranged in pairs. These cells will be found both within the protoplasm of some of the pus cells. The cells containing the gonococcus are usually crowded with the organisms, though the majority of pus cells do not contain them. This organism, on account of its frequent arrangement in pairs, is often called the diplococcus of gonorrhea. It is always found in gonorrheal pus and often persists into the convalescent stage after the external discharge has disappeared. It is easily detected in the pus of acute invasion, while in the subacute and chronic conditions its detection is often a matter of considerable difficulty.

Cultivation.—It does not grow upon the ordinary culture media, and can only be isolated in culture through the employment of special methods. Blood or blood-serum is a necessary constituent of all media for the artificial cultivation of this organism. Some investigators have been successful in growing it upon other body fluids such as ascites fluid, pleural effusion, and the fluid from ovarian cysts. A useful medium may be prepared by mixing equal parts of human blood-serum with ordinary sterilized nutrient agar-agar. This is accomplished by

liquefying the agar maintaining it at a temperature of 50° C. until after the mixture is made, after which it is allowed to solidify.

Distinguishing Characteristics.—1. It is seen practically always in the form of a diplococcus, having the characteristic biscuit form with the long diameters of the individual cells apposed.

2. In gonorrheal pus some organisms are practically always found within the protoplasm of some of the cells.

3. It stains readily with the ordinary staining reagents, but it is promptly decolorized by Gram's.

4. It fails to develop on the ordinary artificial media (separating it from the diplococcus intracellularis meningitidis which grows freely).

5. It has no pathogenic properties for the lower animals.

STAINING.—The ordinary stains are satisfactory, one of the simplest being a 1- or 2-per-cent. aqueous solution of methylene-blue which gives a very clear picture.

Most important as a differential method is its failure to retain its color when treated by Gram's method.

SPECIAL STAINING METHODS.

Gram's Method.

Method.—Objects are first treated with an aniline water solution of gentian-violet which is made after the formula of Koch-Ehrlich (see Appendix for preparation of stain). After staining in this solution for fifteen to thirty minutes the excess of stain is drained off and the film treated for five minutes with Gram's iodine solution (see Appendix). They are next transferred to alcohol and thoroughly rinsed. If careful examination at this point reveals any violet color in the film, it must again be treated with the iodine solution until all violet color is removed. After a final washing in alcohol the specimen may be mounted and examined or a counter-stain of carmine may first be employed.

Wright's Modification.—Stain for one minute in carbol-gentian-violet (see Appendix). Wash in water from thirty

to sixty seconds. Lugol's solution is then allowed to act upon the specimen for one to three minutes. Wash and dry. Differentiate with aniline-xylol (2:1) to which 1.5 per cent. of acetone has been added, for one or two minutes. Wash with xylol, dry, and counter-stain with dilute carbol-fuchsin (1:10) for about one minute, during which the specimen should be warmed slightly. The specimen is finally washed, dried, and mounted for examination.

The process of decolorizing is only a relative one, some bacteria decolorizing more readily than others, so that much depends upon the intensity of the decolorizing reagent, and also upon the time during which it is allowed to act. The counter-stain method with dilute carbol-fuchsin is a differentiated process indicating those organisms that have been decolorized. All decolorized organisms by this method take on a red color. This counter-stain is of particular value where pictures of a number bacteria are made, as in sputum examination.

The following organisms retain the violet stain by Gram's methods:—

Streptococci.
Staphylococci.
Bacillus tuberculosis.
Bacillus anthracis.
Bacillus aërogenes capsullatus.
Bacillus diphtheriæ.
Diplococcus meningitidis intracellularis.
Diplococcus pneumoniæ (Frankel's).
Bacillus tetanus.

The following organisms are decolorized by Gram's method:—

Bacillus pyocyaneous.
Micrococcus gonorrhea (Neisser).
Bacillus malignant œdema.
Bacillus influenza.
Bacillus typhosis.
Bacillus cholera.
Bacillus pneumonia (Friedlander's).

Loeffler's Method of Staining Flagella.

It is essential that the bacteria be evenly and not too numerously distributed over the cover-slip.

Preparation of Cover-Slip.—The glasses must be perfectly clean. Lay six cover-slips on an even surface, and place in the center of each a small drop of distilled water. From the material to be examined, transfer a minute quantity to the drop of water on the first cover-slip; from this one transfer a minute quantity to the second, and so on to the sixth. This insures a varying dilution of the organisms in the different preparation. They are then all spread, dried, and fixed in the usual way. The cover-slip preparations are next warmed in Loeffler's mordant (see Appendix).

A few drops of this solution is placed upon the preparation which is held over a low Bunsen flame until it begins to steam. It should not be boiled. After steaming for a few moments the mordant is washed off with water and then with alcohol. The bacteria are now stained in the Koch-Ehrlich aniline water-fuchsin solution (see Appendix).

When treated in this way various bacteria behave differently, the flagella of some staining readily, others require the addition of an alkali in varying proportion to obtain the best results, others again stain best after the addition of an acid.

To meet these conditions an exact 1-per-cent. solution of caustic soda in water must be prepared, and also a solution of sulphuric acid of such strength that 1 cubic centimeter will exactly neutralize 1 cubic centimeter of the alkaline solution.

For the different bacteria which have been studied by this method Loeffler recommends that one or the other of these solutions be added to the mordant before using, in the following proportions:—

Of the Acid Solution.

For Spirillum concentricum, no addition of either acid or alkali.

For Spirillum cholera Asiaticæ, ½ to 1 drop to 16 cubic centimeters of the mordant.

For Spirillum Metchnicovi, 4 drops of acid to 16 cubic centimeters of the mordant.

For Spirillum rubrum, 6 drops of acid to 16 cubic centimeters of the mordant.

For Bacillus pyocyaneus, 5 drops of acid to 16 cubic centimeters of the mordant.

Of the Alkaline Solution.

For Bacillus mesentericus vulgaris, 4 drops of alkali to 16 cubic centimeters of mordant.

For Bacillus Micrococcus agilis, 20 drops of alkali to 16 cubic centimeters of mordant.

For Bacillus typhosus, 22 drops of alkali to 16 cubic centimeters of mordant.

For Bacillus subtilis, 28 to 30 drops of alkali to 16 cubic centimeters of mordant.

For Bacillus malignant edemæ, 36 to 37 drops of alkali to 16 cubic centimeters of mordant.

For Bacillus symptomatic anthrax, 35 drops of alkali to 16 cubic centimeters of mordant.

The drops used run 22 to the cubic millimeter.

STERILIZATION.

General Considerations. — Acquaintance with the fundamental principles of sterilization and of disinfection are absolutely necessary to the successful performance of all bacteriologic investigations. The term *sterilization*, as commonly employed, implies the absolute destruction of bacterial life by heat, while the term *disinfection* is commonly applied to accomplishing the same end through the agency of chemical substances capable of destroying bacterial life.

Strictly speaking, the term sterilization implies the complete destruction of the vitality of all micro-organisms that may be present in or on the substance or substances to be sterilized. Such a result can obviously be accomplished by either thermal or chemical means, while disinfection need not, of necessity, destroy all living organisms that are present, but only those having the power of infecting or of producing disease, and may or may not, as the case may be, cause complete destruction of bacterial life, as in sterilization. It is therefore possible to accomplish both disinfection and sterilization by either chemical or thermal means.

In the laboratory the employment of these different terms depends upon and is governed by circumstances. It is, of course, essential that all culture media should not only be absolutely

free from all bacteria, whether they are pathogenic or not, but also from their spores. In a word, they must be sterile. At the same time it is equally essential that the original chemical composition and physical properties of the media should remain unaltered by the process. It is self-evident, therefore, that sterilization of such substances by means of chemical agents, is out of the question, for while this method would destroy all bacterial life, it would not only alter the chemical composition of the media, but by becoming inseparably mingled with the media, would, by its continued presence, effectually prevent the growth of bacteria in the material for all time; that is to say, after having performed its function as sterilizer, it would by its continued presence exercise its function as an antiseptic, and render the material useless as a culture medium. Exceptions to this general rule are found in certain volatile substances such as alcohol and ether which, after having performed their bactericidal powers, may be completely driven off by the application of heat.

Sterilization by Heat.—Sterilization by means of high temperatures may be accomplished in a variety of ways: 1. By dry sterilization, which is accompanied by subjecting the articles to adequate degrees of heat in a properly constructed oven. 2. By subjecting them to the influence of live steam at 100° C. 3. By subjecting the substances to steam under pressure. When employing steam under pressure, the temperature to which the articles are subjected will depend upon the pressure developed—the greater the pressure the higher the temperature.

Sterilization by Dry Heat.—This method has the following disadvantages which limit its applicability: 1. The temperature must be relatively high and the period of exposure long as compared to moist heat (steam). 2. The penetration of dry heat into substances to be sterilized is much less thorough than that of steam. 3. Many substances of vegetable and animal origin are rendered valueless by the temperature required for dry sterilization.

Successful sterilization by dry heat cannot usually be accomplished by a temperature lower than 150° C., and this temperature must be continued for not less than an hour. In general, it may be said that dry sterilization is only suited for sterilization of such substances as glassware, dishes, flasks, test-tubes, pi-

pettes, etc., and for such metal instruments as are not injured by heat.

Steam or moist heat sterilization possesses great penetrating power, and is much more rapid and thorough than the above method, and, further, it is far less likely to destroy the material so treated. This method should be employed for sterilizing all culture media, fabrics, cotton, wood, and organic material in general.

Aside from the relative applicability of the two methods, their mode of action toward the organisms to be destroyed is very different. The penetrating power of steam renders it far more efficacious than dry heat. The spores of several organisms which are destroyed by exposure to steam for a few minutes, resist the destructive action of dry heat at a higher temperature for a longer period of time.

The method of applying heat for sterilization depends chiefly upon the character of the substances to be sterilized. The application of dry heat is always continuous, *i.e.*, the objects to be sterilized are simply exposed to the proper temperature for the requisite time necessary to destroy all living organisms and their spores which are either in or upon them. With steam, on the other hand, the articles to be sterilized are frequently of such a nature that prolonged sterilization would injure them. For this reason it has been found desirable to subject such objects to the influence of steam intermittently for a number of short periods.

The PRINCIPLE involved in the intermittent method depends on the differing powers of resistance to heat displayed by different organisms in different stages of their development. During the life of many bacilli they enter a stage during which their resistance to both chemical and thermal agents is materially increased. This increased power of resistance is possessed by the organisms when they are in the spore or resting stage. Some spores of certain organisms have been encountered which retain the power of germination after an exposure of more than an hour to the temperature of boiling water. This difference in the thermal heat-point of bacteria and their spores is taken advantage in the process of sterilization known as the fractional or intermittent method.

As all culture media depend for their usefulness upon more or less unstable organic compounds, the effort of sterilization is to destroy the organisms in the shortest possible time by exposure to least possible amount of heat. This is accomplished by subjecting them to a temperature at a time when the bacteria are in the vegetative or growing stage. In order to develop any existing spores the media, during the intervals between sterilization, should be kept under such conditions of temperature and moisture as will favor the process of vegetation (room-temperature).

During the first application of heat the mature vegetative forms are destroyed, while certain spores which may be present resist this treatment and survive the temperature. Now the sterilization is discontinued and the media is allowed to remain for a time, usually twenty-four hours at room-temperature. During this time those spores which resisted the first heating have conditions favorable to germination. A second short exposure kills this crop of bacilli, when a second rest, followed by a third short exposure, kills the remaining organisms and the media will usually be found sterile.

It should be remembered that while all spores which are present are not killed by the first exposure, still their power of germination may be so inhibited by the exposure to 100° C. that their germination is delayed, that they cannot possibly germinate during the twenty-four hours' intervals.

Experiment has shown that the fractional process gives the best results when the objects are subjected to the action of live steam (steam at ordinary atmospheric pressure) for fifteen minutes on each of three consecutive days, and that during the intervals the cultures should be maintained at a temperature between 25° and 30° C. The substances thus treated will remain sterile for an indefinite time, provided they are not exposed to the re-entrance of micro-organisms.

An occasional exception will be noted when, after careful treatment as above outlined, certain species of spore-forming bacteria will not have been entirely destroyed by this method. These are usually of the non-pathogenic group of the so-called soil organisms.

Finally it must be born in mind that this method is only

applicable to substances capable of presenting conditions favorable to spore germination, and that dry substances, such as instruments, apparatus, or organic materials, in which decomposition has set in, where the natural conditions favorable to spore germination are absent should not be treated by this method, but must be subjected to higher temperatures for longer periods of time.

Intermittent Sterilization (at low temperature). — The process of intermittent sterilization at comparatively low temperatures is based upon the principle outlined above, but differs in the details of its application as follows: 1. It requires a greater number of exposures to accomplish complete sterilization. 2. The temperature at which it is accomplished is not above 68°-70° C.

It is employed for sterilization of easily decomposible materials and those which would be rendered useless by the application of steam at 100° C., but which are unaltered by the temperature employed. This method is applicable for sterilization of certain albuminous media where it is desirable to retain their fluid condition during sterilization and which would be coagulated by exposure to higher temperature.

This process requires that the temperature employed should be between 68°-70° C., and that an exposure of one hour should be made each of six consecutive days. During the intervals the material is kept at a temperature between 25°-30° C. to favor germination of any spores that may be present.

Direct Steam Method.—Sterilization by means of steam is also accomplished by what is known as the direct or continuous method. By this process both mature organisms and their spores are destroyed by a single exposure to steam at zero pressure—live or steaming steam. The sterilization is accomplished by a single exposure of one hour.

Steam-Pressure Method (by Autoclave). — By employing steam under pressure we are able, by increasing the temperature, to materially shorten the time necessary to accomplish complete sterilization. By employing a pressure of approximately one atmosphere (15 pounds) a temperature of about 122° C. is obtained. This is sufficient to accomplish complete sterilization by one exposure of fifteen minutes.

When this method is employed it will occasionally be found that the coagulating power of gelatin is reduced, and that it becomes slightly cloudy, while in agar-agar a fine, flaky precipitate is noticed. For accurate time and temperature exposures this is a very uncertain method. Obviously the material is subject to active temperatures during the heating up as well as during the cooling process, besides the actual time during which the maximum pressure is maintained. Also, if great care is not

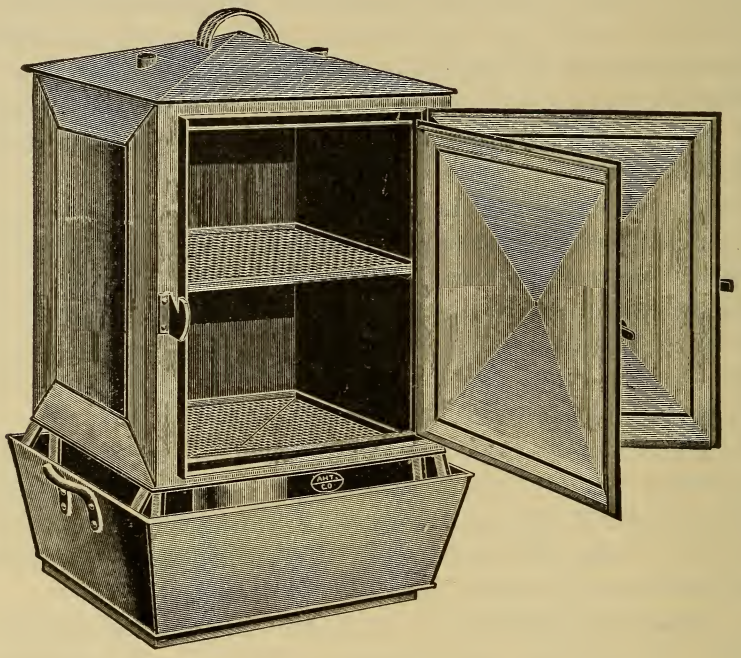


Fig. 31.—Arnold Steam Sterilizer. (A. H. Thomas.)

observed to prevent premature opening of the autoclave, a sudden, rapid ebullition of the fluid occurs which, in the case of test-tubes, the media may completely boil away.

While this method of sterilization is not well suited for delicate experiments where a definite time exposure to definite temperature is of importance, still, for general laboratory purposes, it has much to recommend it in the way of a time saver, and in the certainty with which sterilization is accomplished. The apparatus designed to sterilize under pressure is termed an autoclave.

Practical Application of the Method. — 1. The Arnold Steam Sterilizer (Fig. 31): This is probably the best sterilizer for general laboratory purposes, since it is simple and economic in its operation. The difference between this apparatus and the original sterilizer devised by Koch is that it provides for the condensation of the steam after its escape from the

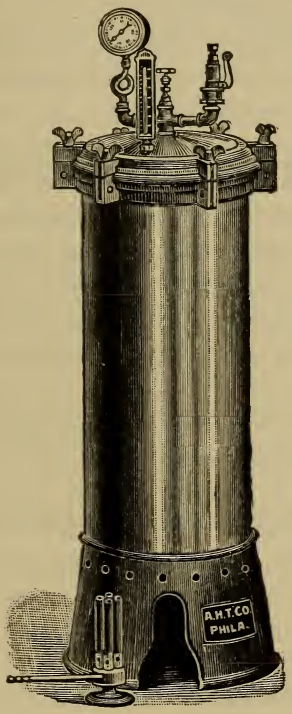


Fig. 32.—Autoclave. (A. H. Thomas.)

sterilizing chamber, and returns the water of condensation automatically to the reservoir.

2. The Autoclave.—The advantage of this method is so well recognized that its use has practically superseded the intermittent method with live steam, except when the temperature developed by the autoclave is sufficient to destroy the materials subjected to it. By this plan sterilization is accomplished in fifteen minutes by exposure to steam under the pressure of one atmosphere.

The autoclave (see Fig. 32) embodies the same principle as the steam sterilizer. It provides for the generation of steam within a chamber capable of being hermetically sealed after the introduction of the substances to be sterilized. The chamber is fitted with a safety-valve arranged for regulating the degree of pressure. A thermometer passes through the wall and enters the chamber, thus allowing the temperature to be followed and the pressure properly regulated.

Hot-Air Sterilizer.—The hot-air sterilizers employed for this work are simply double-walled boxes of Swedish iron, having a double door and a copper bottom. They are fitted with proper openings in the walls to permit circulation of the heated air. The heat is obtained from the flame of a Bunsen burner applied directly to its bottom. These bottoms are usually constructed of copper, and because they readily burn out, are now made so that they may easily be replaced. Properly constructed sterilizers with removable bottoms may now be obtained and should be sought when purchasing, as they will save much annoyance and not a little expense.

Sterilization by this method is accomplished in from a half to one hour at a temperature of from 150°-180° C.

CHEMICAL STERILIZATION AND DISINFECTION.

It is possible by means of certain chemical substances to destroy all bacteria and their spores, or by the same means to remove all their pathogenic properties; in other words, to disinfect them.

When it is desirable to use chemical disinfection in the laboratory, successful results may be obtained by employing a 33- to 40-per-cent. solution of carbolic acid. Under ordinary circumstances this will accomplish the result in from twenty minutes to half an hour. It is, however, not reliable for the destruction of spores of resistant organisms, such as the spores of the anthrax bacillus.

All materials and issues containing infectious organisms should be burned and all other cloths, test-tubes, flasks, dishes, etc., should be boiled in a 2-per-cent. soda (ordinary washing soda) for a half-hour, or should be exposed in the steam sterilizer for the same length of time.

Intestinal evacuations are best disinfected with chlorinated lime, which should contain at least 0.25 per cent. of free chlorin. This solution should be mixed in equal parts with the material to be disinfected, and then should be allowed to stand for one or two hours before being disposed of.

Sputum in which tubercle bacilli may be present, as well as vessels containing it, and the eating utensils of tuberculous patients, should be boiled with a 2-per-cent. soda solution for from a half to one hour, or should be exposed in the steam sterilizer for the same period.

PREPARATION OF CULTURE MEDIA.

Bouillon.—Five hundred grams of freshly chopped, lean beef, free from fat and tendons, are soaked in a liter of water for twenty-four hours, during which time the temperature of the mixture is kept low by surrounding it with ice.

At the expiration of this time the mixture should be strained through coarse muslin until a liter of fluid has been recovered. To this now add ten grams of dried peptone and five grams of common table salt. It is then to be rendered neutral or slightly alkaline by the addition of a few drops of a saturated sodium carbonate solution. The flask containing the mixture is then placed either in the steam sterilizer or upon a water-bath and kept at the boiling point until all the albumin has been coagulated, and the fluid portion is clear and of a pale-straw color. It is then filtered through a folded filter-paper and finally sterilized in the steam sterilizer by the fractional method (see page 259). This is the original method of Koch which has been modified and improved in the following ways:—

Neutralization.—Ordinarily this is accomplished by the addition of a saturated solution of sodium carbonate, and the reaction determined by red and blue litmus paper. This sodium carbonate solution is not so good, however, as a strong solution of sodium or potassium hydroxid, because the carbonic acid arising during the process of neutralization with the sodium carbonate frequently produces a temporary acid reaction which later disappears on boiling. Exact titration with an 0.4-per-cent. solution of sodium hydrate, obviates this difficulty. The process

is applied only after the bouillon has been deprived of all its coagulable albumin by boiling and has again been reduced to room-temperature.

Technic.—First ascertain the exact volume of the fluid. From this sample take exactly 5 or 10 cubic centimeters and add a few drops of a 1-per-cent. alcoholic solution of phenolphthalein as an indicator. The 0.4-per-cent. alkaline solution is placed in a graduated burette, and the solution to be tested in a porcelain dish or casserole. Now add the alkaline solution, drop by drop, until the bouillon turns a faint rose color. A second measured quantity of bouillon is treated in the same manner as a check, and if the amount of sodium hydrate solution required to cause neutralization is the same or only slightly different, a simple calculation will indicate the amount of soda solution required to neutralize the bulk of the medium. Thus, if for 10 cubic centimeters of the bouillon would be required 1.5 cubic centimeters of the 0.4-per-cent. solution of sodium hydrate, then for the remaining 980 cubic centimeters (original volume 500 cubic centimeters), it would require 98 times 1.5 cubic centimeters of the soda solution to neutralize the total amount of bouillon, or 147 of the 0.4-per-cent. sodium hydrate solution would have to be added to the 980 cubic centimeters of bouillon to accomplish neutralization.

To avoid over-diluting the bouillon by the weak alkaline solution, it is better to employ for this purpose a 4.0-per-cent. solution of NaOH, of which only 14.7 cubic centimeters would be required.

Not infrequently the filtered neutralized and sterilized bouillon will be found to contain a fine flocculent precipitate. This may be due either to an excess of alkalinity or to incomplete precipitation of the albumin. The former may be corrected by the addition of a little dilute acetic or hydrochloric acid, follower by a second boiling, filtering, and sterilization. If due to imperfect precipitation of the albumin this may be corrected by reboiling and filtering.

2. The substitution of prepared meat extract for the fresh extract is now almost universal. Any good stock meat extract will answer the purpose, and should be used in the strength of from two to four grams to the liter of water. Peptone and

sodium chlorid are added, as in the original method of preparation.

The advantages of the meat extract are a decided shortening of the time required for preparation of the media, and the production of a more uniform material.

Nutrient Gelatin.—In making nutrient gelatin the bouillon is prepared first as outlined above, except that the reaction is corrected only after the gelatin has been completely dissolved. The reaction of the gelatin of the shops is frequently quite acid, entailing the addition of considerable more alkaline solution than required for the bouillon alone.

The gelatin is added in sufficient quantity to make a 10- or 12-per-cent. solution. Its complete solution is accomplished either on a water-bath or over the bare flame. In the latter instance it must be constantly stirred to prevent burning the gelatin on the bottom of the pan. When the gelatin is completely dissolved it can readily be filtered through an ordinary folded filter paper in a glass funnel. It not infrequently happens that in spite of the most careful technic the filtered gelatin is not perfectly clear; then clarification is required. For this purpose the mass must be redissolved, and when the temperature is between 60° and 70° C. the white of an egg, which has been beaten up with about 50 cubic centimeters of water, is added and thoroughly mixed in. This mixture is then again brought to the boiling point until coagulation of the egg albumin occurs. This process results in large, flaky coagula of albumin, which it is best not to break up, as fine flakes of albumin will clog the filter-paper and materially interfere with the process of filtration.

The filter-paper should always be moistened before filtering. If this is not done, the pores of the filter-paper will become clogged with gelatin and coagulated albumin, which will greatly interfere with rapid filtering.

Gelatin should not, as a rule, be boiled for more than fifteen minutes, nor left in the steam sterilizer for more than thirty minutes, otherwise its property of solidifying will be impaired.

As soon as the preparation of the gelatin is completed it should be sterilized in the steam sterilizer for fifteen minutes on

three consecutive days. The mouths of the containing flask or test-tubes should be completely plugged with raw cotton.

Nutrient Agar-Agar.—The preparation of this is difficult and tedious, and frequently fails from lack of patience or of experience, or both. Every worker has some slight modification of his own, but if, according to Abbott, the following directions are carefully carried out, the product will usually be satisfactory.

Prepare bouillon in the usual way. As agar-agar reacts neutral or faintly alkaline, the neutralization of the bouillon may be accomplished before the agar-agar is added. Finely chopped or powdered agar-agar is added to the bouillon in the proportion of 1 to 1.5 per cent. The mixture is then placed in an agate or earthen-ware boiler, and the height of the fluid, before boiling, marked upon its inside. If a liter of the medium is being made add about 275 cubic centimeters or more of water, and boil slowly for about two hours or until the excess of water that was added has been evaporated. Do not allow the fluid in the vessel to fall below the original level. If this occurs water must be added to bring the final amount up to one liter. At the expiration of two hours remove from the fire and cool rapidly by immersion in a pan of cold water. Stir constantly until the temperature of the mass has fallen to about 70° C., then add the white of one egg that has been previously beaten up in about 50 cubic centimeters of water. Mix this in well and allow to boil for half an hour longer, keeping the fluid up to the original one liter mark. The fluid is now easily filtered at room-temperature through a heavy folded filter. If properly prepared and filtered through a properly folded filter-paper, it should pass through at the rate of one liter in from fifteen to twenty minutes.

Glycerin Agar-Agar.—The nutrient properties of agar-agar for certain organisms is greatly increased by the addition of 5-per-cent. glycerin. If glycerin is added to the agar-agar, it should be done after filtration and before sterilization.

If after filtration the medium is found to contain flocculi, investigate the reaction. If it is quite alkaline it must be neutralized, boiled, and filtered again. If the reaction is neutral

or only faintly acid, dissolve and again clarify with egg albumin as directed above.

The most important feature of all media apart from their proper preparation is the reaction. They must be neutral or only very faintly alkaline to litmus, as but few organisms develop well on acid media.

Blood-Serum.—For the preparation of a small amount of blood-serum for culture media purposes, it may be obtained from small animals under such aseptic precautions as will guard against gross contamination. For laboratory purposes, where large amounts are required, it is best obtained from the slaughter houses. Under these conditions a certain amount of contamination is unavoidable, though its extent may be limited by observing certain precautions.

The original method of Koch with a few slight variations is as follows:—

The animal from which the blood is to be obtained should be suspended by the hind legs so that its head is a few feet from the floor. The head should be held back when, with one sweep of a sharp knife, the throat is completely cut through. The blood as it spurts from the vessels should be collected in large glass jars which have been previously sterilized and dried with alcohol and ether. The jars should be provided with close-fitting cover and clamps capable of hermetically sealing them (large museum specimen jars will be found very satisfactory for this purpose). From two-gallon jars of blood there is usually recovered from 500 to 700 cubic centimeters of clear serum.

The jars having been filled with blood, their covers are replaced loosely and then they are allowed to stand quietly for about twenty minutes until clotting has begun. At the expiration of this time a clean glass rod is passed about the edges of the surface of the forming clot to break up any adhesions that have formed to the sides of the jar. The covers are now replaced and clamped down tightly, then with as little agitation as possible the jars are transferred to an ice chest where they should remain for from twenty-four to forty-eight hours. The temperature of the ice chest should be only sufficiently low to prevent bacterial growth, but not so cold as to prevent coagulation. When the jars are removed from the ice chest a firm clot

will be found in the bottom of the jars. The serum is drawn off with a sterile pipette or syphon, and transferred to sterile glass cylinders. These cylinders are now placed on ice for another twenty-four hours, when the corpuscles will have settled to the bottom, leaving the serum above quite clear. This is then ready to be pipetted off into sterile test-tubes, about 8 cubic centimeters in each tube, or into flasks of 100 cubic centimeters' capacity. It is now ready for sterilization. This is accomplished by the intermittent method at a temperature of 70° C. for a period of one hour on five consecutive days. During the intervals it should be kept at room-temperature. After sterilization the tubes may be allowed to remain fluid or they may be coagulated by a short exposure to 80° C. For solidifying, the tubes should be placed in an inclined position in order to secure the greatest possible surface from the quantity of serum employed.

The process of solidification requires constant attention if good results are to be obtained. No rule can be laid down for the time required to accomplish it, as this is not constant. Too high temperature and too rapid solidification results in an opaque and inelastic medium.

When solidification is complete the tubes may be retained in a vertical position, and unless for immediate use must be protected from drying. This may be done by burning off the superfluous ends of cotton and covering them with sterile rubber caps; or what is just as satisfactory and far cheaper, sterilized corks may be pushed down upon the cotton plugs.

Owing to the employment of large quantities of serum, principally for the detection of diphtheria, the tedious method of Koch has been largely superseded by a number of more rapid modifications.

Method of Councilman and Mallory.—By this method the serum is more quickly prepared. Rigid precautions against contamination of the blood during collection are not necessary, and the resulting medium, while neither transparent or translucent, fully meets the ordinary requirements of bacteriology.

By this method the serum is decanted directly into sterile test-tubes as soon as obtained; it is then firmly coagulated in the slant position by exposure in a dry-air sterilizer at from 80° to 90° C. It is then immediately sterilized in the steam sterilizer

at 100° C. for fifteen minutes on three consecutive days, as is the case with other media.

Loeffler's Blood-Serum Mixture.—This mixture consists of one part neutral meat-infusion bouillon, containing 1 per cent. of grape-sugar and three parts blood-serum. The mixture is placed in test-tubes, sterilized, and solidified in exactly the same manner as described under blood-serum preparation, except that it requires a longer time and a higher temperature for coagulation.

PREPARATION OF TUBES, FLASKS, ETC., FOR CULTURE MEDIA.

While the media are in the course of preparation it is well to get the tubes, flasks, pipettes, etc., ready for their reception. These must be absolutely sterile. To this end both old and new tubes should first be boiled for a half-hour in a 2-per-cent. soda solution, then carefully swabbed out with appropriate bristle brushes. After rinsing in clear water they are immersed in a 1-per-cent. solution of hydrochloric acid for a few minutes, then rinsed again and stood, round end up, to drain. When dry they are plugged with raw cotton carefully inserted so that there are no cracks or openings in the occluding cap. The plug should fit neither too loosely nor too tightly, but should fit firm enough to hold the weight of the tube when lifted by the protruding cotton.

The tubes thus plugged are then placed upright in wire baskets and heated for one hour in the hot-air sterilizer to a temperature of 150° C. Tubes so prepared, if undisturbed, will remain sterile for an indefinite period.

Filling Tubes.—The tubes are best filled with the aid of a separating funnel, though if not convenient the tubes can, with a little care, be successfully filled directly from the flasks. It is not necessary to sterilize the funnel as the media in the tubes is to be sterilized as soon as they are filled. In any case, care should be observed to prevent any of the medium from coming in contact with the mouth of the tubes, which would cause the cotton plugs to adhere to it, making them hard to remove, presenting a very untidy appearance and materially interfering with subsequent manipulations.

After filling, the tubes are ready for final sterilization. This

is accomplished in the steam sterilizer by the three-day fractional method.

TECHNIC FOR PLATES AND PETRI DISHES.

Plates.—The plate method can be employed with both agar and gelatin, but cannot be practiced with blood-serum, because the latter when once it is solidified cannot again be rendered liquid.

Plates are usually referred to as a set. This term includes three separate plates each representing a mixture of the organism in a state of greater dilution. The plates are numbered 1, 2, and 3. A set of plates may be prepared as follows: Three tubes, each containing the requisite amount of gelatin or agar-agar, are placed in a water-bath and warmed until the medium is fluid. Agar-agar becomes fluid at about the temperature of boiling water; gelatin is fluid between 35° and 40° C. In the case of the agar-agar tubes, after liquefying they must be cooled to 40° C., at which temperature they remain fluid while the organisms are introduced. If this cooling is omitted the temperature of the medium when the organisms are inoculated will be sufficiently high to destroy their vitality.

The medium now being liquid and of a proper temperature, the material containing the organisms is taken up on a sterile platinum wire-loop and transferred to tube 1, where it is thoroughly disintegrated and mixed by rubbing against the side of the tube. The more carefully this is done the more uniform will be the distribution of the organisms and the better the final results. The loop is now again sterilized by passing through the flame, and when cool three loops full from tube 1 are transferred to tube 2, where they are carefully stirred in. Again, the wire is sterilized and the same manipulation carried out between tubes 2 and 3. This completes the dilution.

During these manipulations, which must be done rapidly if agar is employed, the temperature of the water bath must be kept between 39° and 43° C. If the temperature falls below 38° C. the agar-agar will become solidified and can then only be reliquefied by the application of heat sufficient to destroy the organisms introduced.

After inoculation the contents of these tubes are poured out

upon the sterilized plates, cooled, and incubated for twenty-four or forty-eight hours.

Petri Dish Method.—This process materially simplifies the original technic of the plate method. It consists in substituting for the flat plates of glass small, round double-glass dishes having about the same surface area as the original plates. The inoculated and liquid media is poured directly into these, their covers are immediately replaced, and they are set aside to cool. In all other respects the process is the same as Koch's original plate method. These dishes have vertical sides which prevents overflowing of the medium. A convenient size for this method measures about 12 centimeters in diameter, but dishes of other sizes are easily obtainable. The dishes are readily sterilizable by hot air or steam, and have the great advantages that the danger of contamination is reduced to a minimum, since after sterilizing the plates do not have to be separated until the pouring on of the medium, and then only for a moment.

THE INCUBATOR.

After the plates have been made and solidified they should be transferred to an incubator where a uniform and favorable temperature may be maintained. Various types of incubators have been devised, but since the principle and purpose of all is the same, a general description of their construction and method of employment is all that is required.

The incubator or thermostat (Fig. 33) consists essentially of a copper chamber of convenient size provided with double walls, between which heated water circulates. The incubator chamber has a close-fitting door of heat-proof construction, and usually within this is a second door provided with a glass front, which permits inspection of the interior of the incubator without actually opening the chamber, and so reducing the temperature. The whole apparatus is set upon an enclosed base and is covered with asbestos board to prevent loss of heat from radiation. In the top of the chamber is a small opening fitted with a perforated cork through which a thermometer projects into the interior. Two other openings are provided, one for the thermometer which records the temperature of the circulating water, the other for the thermo-regulator. At one side of the

apparatus is a vertical water gauge provided with an upper opening for the introduction of water, and a stop-cock below for drawing off the water when occasion required.

When in operation the apparatus should be kept full of water, otherwise the object of the water jacket will be defeated and the temperature of the interior of the chamber will not be maintained. Heat is supplied to the incubator by a gas burner

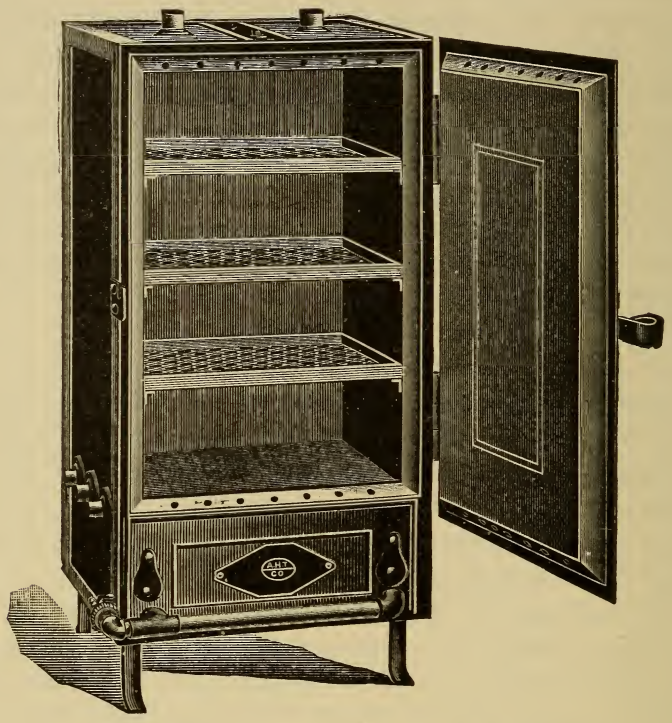


FIG. 33.—THERMOSTAT OR INCUBATOR. (A. H. THOMAS.)

placed within the inclosed space below the chamber. The particular form of burner usually employed is known as "Koch's safety burner," which is so constructed that should by accident the light be extinguished, the flow of gas would be almost immediately shut off. An ordinary Bunsen burner, well protected from sudden gusts of air, will serve the purpose equally as well.

The Thermo-Regulator.—The efficiency of the thermostat depends upon the proper and uniform temperature which is

maintained by the thermo-regulator. A satisfactory regulator should permit of a fluctuation of not more than 0.2° C. in the temperature within the chamber of the apparatus.

The commonest form of regulator is constructed upon the principle involving the expansion and contraction of fluids under the influence of heat and cold. By means of such expansion and contraction the amount of gas passing from the source of supply to the burner is modified, as the temperature of the water in the incubator rises or falls.

For the successful employment of clinical laboratory methods, the thermo-regulator is useful but not essential. In a room of fairly uniform temperature the flow of gas can be regulated by hand until the internal temperature of the incubator is 37° C. After this, if the water level is maintained and the apparatus protected from protracted changes in the surrounding temperature, the variation in the internal temperature will be so slight that it need not be considered.

XVI.

APPENDIX.

DESCRIPTION OF OFFICE LABORATORY CABINET.

For those who are daily employing the methods of clinical medicine a detailed description of the author's laboratory cabinet may be of interest. As illustrated in frontispiece, this cabinet is constructed of white pine or poplar, of half-inch boards, except the lowest shelf or table which is one-inch in thickness. This can easily be constructed by a carpenter and finished in white enamel.

Its adoption will be found particularly useful to those not fortunate enough to have a room to devote to laboratory purposes. The cabinet itself is not at all unsightly, and may be fastened to the wall in the consulting room, preferably near a washstand, as running water should be easily accessible when possible. For those who possess the advantage of a well-equipped laboratory, it will be found extremely convenient for preliminary rough examinations of specimens as soon as they are received.

The cabinet occupies a wall space of 22 ins. broad by 28 ins. high, and projects outward from the wall 18 ins. The shelves are 6½ ins. deep, the top shelf providing space for a gallon bottle of distilled water with syphon-tube attachment, and place for storage of large stock reagent bottles, beakers, flasks, dishes, etc. The compartment furnished with a glass door is for the microscope and accessories, where they may be safely kept under lock and key.

The shelf below the top provides space for 24 2-ounce reagent bottles or a less number of larger ones. The drawer immediately below this holds filter paper, Petri dishes, forceps, short pipettes, etc., while the small compartment to its right is

very convenient for a few test-tubes, cover-glasses, slides, and litmus paper.

The table, which measures 18 by 21 ins., is sufficiently large to contain the necessary racks, stands, etc. Passing through the table to its extreme left is a gas-cock with nipple for the attachment of a Bunsen burner.

Such a cabinet will provide all the necessities for the usual examinations of urine, gastric contents, and sputum. By modification and amplification of this idea the cabinet can be adapted to all the special examinations employed in clinical medicine.

EXACT DIMENSIONS OF CABINET.

Total height, 28 ins.; total width, 22 ins.; depth of table, 18 ins.; depth of shelves, 6½ ins.; height of drawer base above

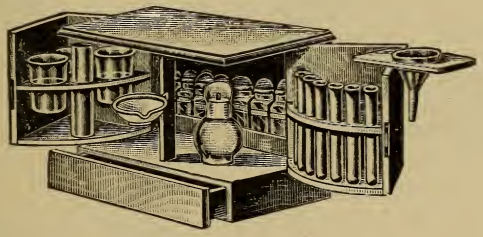


FIG. 34.—PORTABLE URINALYSIS SET.

table, 8 ins.; size of drawer, 6 by 10 by 4 ins.; microscope compartment, 8 ins. wide by 14 ins. high.; height from table to top shelf, 24 ins.

The wood should be carefully selected, free from knots and thoroughly seasoned. It should be joined by screws and glue to prevent warping and separation of the joints and seams. Two coats of good white paint, followed by the same number of white bath-tub enamel, will complete the construction. The door and drawer-knobs are of glass, and the microscope compartment is fitted with a lock.

Since the construction of this cabinet three years ago the following improvements have suggested themselves: A large shallow drawer could be placed under the table. This would

be very useful for storing burettes, large pipettes, etc., and would not materially add to the size of the piece. A wooden burette stand and filter holder could be easily attached to the under surface of the first shelf, and so arranged that when not in use they could be swung under the shelf and out of the way.

Fig. 34 shows a portable urinalysis outfit, which is convenient for bedside work. It may be obtained in the open market at a moderate price.

URINALYSIS.

Report of Clinical Laboratory.

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Physical Characteristics.		Quantitative Determinations.
Quantity in 24 hours.	c.cm. / oz.	Albumin: Heat, 10% Acetic Acid. / Heller's Nitric Acid. / Heat and Nitric Acid.
Color,		Glucose: Fehling's. / Phenylhydrazin. / Böttger's Bismuth.
Appearance,		Indican,
Odor,		Skatol,
Sediment:—		Acetone,
Mucus,		Bile Pigments,
Urates,		Blood,
Phosphates,		Diazo,
Uric Acid,		Aceto-acetic Acid,
Pus,		B-Oxybutyric Acid,
Specific Gravity,		Drug Reactions,
Reaction,		Cammidge Reaction,

Quantitative Determination.

Albumin (Esbach), (Purdy),
Glucose (Fehling's), (Fermentation),
Urea,
Ethereal Sulphates,
Chlorids,

Microscopic Examination.

(Centrifugated),	(Sedimented).
Casts.: Hyaline, / Granular, / Fatty, / Epithelial, / Blood, / Waxy, / Bacterial,	Erythrocytes,
Cylindroids,	Leukocytes,
Mucus,	Pus cells,
	Epithelia,
	Spermatazoa,
	Bacteria,
	Yeast Spores,
	Trichomonides.

Voided, A.M.....P.M..... Examined, A.M.....P.M.....

Examined by .....

SPUTUM EXAMINATION.

Report of Clinical Laboratory.

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Physical Characteristics.

Quantity in 24 hours. } c.cm. / oz.

Color,

Odor,

Consistence,

Blood,

Character,

- Serous,
- Mucous,
- Muco-purulent,
- Purulent,

Casts,

Spirals,

Microscopic Examination.

Tubercle Bacilli,	Blood-Cells,
Micrococcus Lanceolatus,	Pus Cells,
Staphylococci,	"Heart Failure" Cells,
Streptococci,	Hematin Crystals,
Bacilli,	Elastic Fiber,
Actinomyces,	Curschmann's Spirals,
Yeast,	Casts,

Obtained ..... Examined .....

Examined by .....

BLOOD EXAMINATION.

Report of Clinical Laboratory.

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.....

General Examination.

Color,

Coagulability { Estimated, / Wright's, / Bogg's,

Viscosity,
Flow from Puncture,
Specific Gravity,

Erythrocytes { Thoma-Zeiss per c.mm., / Estimated by Daland Hematokrit, / Percent.,

Hemoglobin { Fleischl's Hemoglobinometer, / Sahli's Hemoglobinometer, / Tallqvist's Scale,

Color Index,
Plaques,
Leukocytes,

Microscopic Examination.

Erythrocytes.

Color,
Polychromatophilia,
Granular Degeneration,
Poikilocytosis,
Macrocytes,
Microcytes,
Normoblasts,

Leukocytes.

Differential Count,
- Number of cells counted,
- Polymorphonuclears,
- Large lymphocytes,
- Small lymphocytes,
- Eosinophiles,
- Basophiles,
- Myelocytes,

Parasites,
Bacteria,
Hematin Crystals,

Date .....

Examined by .....

GASTRO-ANALYSIS.

Report of Clinical Laboratory.

.....

.....

Vomitus,
Test meal

Composition:—

Ingested,
Extracted,
Amount Recovered,
Dilution,

Physical Characteristics.

Sediment containing:—

Color,
Odor,
Consistency,
Reaction,

Blood,
Bile,
Mucus,

Chemical Determinations.

Total Acidity,
Free Hydrochloric Acid,
Combined " "
Lactic Acid,
Protealysis,

Butyric Acid,
Acetic Acid,
Blood,
Bile,

Microscopic Examination.

Starch Grains,
Meat Fiber,
Epithelia,
Erythrocytes,
Leukocytes,

Oppler-Boas Bacillus,
Sarcinæ,
Bacteria,
Necrotic Tissue,
Parasites,

Date ..... Examined .....

Examined by .....

BLOOD-PRESSURE DETERMINATIONS.

Clinical Report.

.....

.....

Apparatus { Faught, / Stanton,

Width of Cuff cm.

Part examined,

Right,

Left,

Posture,

Pulse Rate,

Systolic mm.Hg.

Diastolic mm.Hg.

Pulse Pressure mm.Hg.

Mean Pressure mm.Hg.

Remarks.

Time of Day. A. M..... P. M.....

Date .....

Examined by .....

EXAMINATION OF THE FECES.

Report of Clinical Laboratory.

.....

.....

Physical Characteristics.

Number of Stools in 24 hours.
Amount in 24 Hours.
Color,
Odor,
Consistence,
Blood,
Pus,
Mucus,
Parasites,
Crystals,
Calculi,
Connective tissue,
Muscle fiber,
Foreign bodies,

Chemical Examination.

Blood,
Hydrobilirubin (sublimate test),
Fermentation,
"Lost" Albumin,

Microscopic Examination.

Erythrocytes,
Pus cells,
Epithelia,
Crystals,
Meat Fiber,
Connective Tissue,
Starch Cells,
Free Fat,
Fatty acids,
Tubercle Bacillus,
Shiga Bacillus,
Comma Bacillus,
B. Aerogenes Capsulatus,
Amebæ,
Parasites,

Obtained ..... Examined .....

Examined by .....

CLINICAL TERMS.

Owing to the various and uncertain terms employed to designate the amounts of substances found, in clinical investigations the following scheme has been successfully employed in our laboratory.

Terms to be used in expressing the results of examinations of specimens in laboratory[1]:—

Albumin, Sugar, Indican, Acetone, Bile, Blood, etc.	Questionable trace.
	Very faint trace.
	Faint trace
	Trace.
	Strong trace.
	Moderate amount.
	Large amount.
	Very large amount.
	Excessively large amount.

For Sediments use:—

Occasional.

Few.

Moderate number.

Many.

Very many.

Excessively large number.

[1] After Judson Daland.

OFFICIAL REQUEST FOR WIDAL REACTION, COUNTY AND CITY OF PHILADELPHIA, PENNA.

Page 1

TO BE FILLED OUT BY ATTENDING PHYSICIAN.

Is this the first specimen from this case?.....Date.....
Name of Attending Physician.....
Residence .....
Name of Patient.....Age.....
Residence .....Ward.....

All data must be given or report will not be made.
(See inside for Directions.)

Page 2

TO BE FILLED IN BY ATTENDING PHYSICIAN.

Day of fever.....Has patient previously had Typhoid?.....
When? .....Sex.....
Mark any of the following symptoms observed:
Diarrhœa? .....Temp. Range.....Enlarged Spleen?.....
Delirium? .....Iliac Tenderness?.....Rose Spots?.....
State suspected source of infection.....
Clinical Diagnosis and Remarks: .....

Page 3

DIRECTIONS FOR TAKING BLOOD DROP.

Thoroughly cleanse the skin of the patient's finger tip or lobe of the ear. After carefully drying, prick it with a needle previously sterilized by heating over a lamp or gas flame and allowed to cool. Allow **five** or more large drops of blood to dry on the inner surface of the accompanying piece of folded paper. Fill out the first **two** pages of this book. Replace the folded paper and book in the **colored** envelope, which should be stamped and posted or otherwise returned to the Laboratory, Room 708, City Hall.

Page 4

CIRCUMSTANCES INFLUENCING THE INTERPRETATION OF THE RESULTS OBTAINED.

The test is not likely to yield positive results before the fifth or sixth day of an attack of typhoid fever, but the best results are obtained about the tenth day.

The condition of the blood causing the reaction persists for some time after convalescence, hence care must be taken to exclude the coincidence of a positive result due to a previous attack of typhoid fever.

In doubtful cases giving negative reaction, send further samples at intervals of two or three days, so long as there is a possibility of typhoid fever.

Please inform the Laboratory, *by reply postal sent you*, of any discrepancy between result of test and the subsequent course of case.

APPARATUS.

The following apparatus will be found necessary to conduct the examinations described in this book:—

FOR URINALYSIS.

Purette and pinch-cock.
Pipette, 1 c.c.
Pipette, 5 c.c.
Pipette, 10 c.c.
Flask, 1000 c.c.
Flask, 100 c.c.
Graduated cylinder, 50 c.c.
Urinometer and jar.
Ureometer, Doremus.
Albuminometer, Esbach.
Plain glass pipettes.
Glass stirring-rod.
Porcelain dishes.
Porcelain tile.
Beakers.
Sedimentation glasses.
Covers for glasses.
Funnels.
Test-tubes, assorted sizes.
Centrifuge.
Plain centrifuge tubes.
Graduated Purdy tubes.
Bunsen burner and hose.
Tripod.
Wire gauze.
Burette stand.
Filter paper.
Microscope.
Westphal Balance.

FOR BLOOD AND BLOOD-PRESSURE.

Gower's, Fleichl's or Sahli's hemoglobinometer.
Thoma-Zeiss hemocytometer.
Drop-bottles and droppers.
Hypodermic syringe.
Faught or Stanton sphygmomanometer.
Daland lancet.
Microscope and accessories.
Microscope slides.
Cover-glass, 7/8 in. diameter.
Mechanical stage.
Spectroscope.
Wright's blood-coagulation apparatus or Bogg's modification of Russell and Brodies' apparatus.

FOR STOMACH EXAMINATION.

Two Mohr's burettes (25 and 50 cubic centimeters) will be required.
Dimethyl-amido-azobenzol paper.
Dimethyl-amido-azobenzol (Topfer's reagent).

Deci-normal sodium hydrate.
Phenol-phthalein (1 per cent. alcoholic).
Sol. neutral ferric chlorid.
Congo-red paper.
Starch paper.
Powdered pepsin.
Sodium carbonate.
Potassium hydrate, 10 per cent. sol.
Egg albumin in cubes or buttons preserved in glycerin.
Mett's Capillary Albumin Tubes.

FOR FECES, CEREBRO-SPINAL FLUID AND MILK.

Stool sieve.
Breast-pump.
Hydrometer, 1010 to 1040.
Graduated cream-gauge.
Cream centrifuge tube and pipettes.
Capillary tube, heavy glass. Diameter, 0.05 mm. Length, 800 to 1000 mm.

FOR BACTERIOLOGIC AND OPSONIC WORK.

Incubator.
1 pr. balances accurate to 0.2 gram.
Bunsen burner.
Thermometer registering 200° C.
Thermometer graduating in tenths. Registering from 0 to 50° C.
Large watch-crystals.
Pure culture of typhoid bacillus.
McFarland & L'Engle's nephelometer.
Arnold steam sterilizer.
Thermo-regulator.
Rubber tubing.
Platinum wire loops, glass handle.
Sterile bouillon in tubes.
Agar slants.
Capillary pipettes.
Hanging drop slides.

CHEMICALS AND REAGENTS.

The following chemicals and reagents will be required to carry out the examinations outlined in this work:—

CHEMICALS—FOR URINALYSIS.

Acid, nitric.
sulphuric.
hydrochloric C. P.
hydrochloric deci-normal.
acetic, glacial.
acetic, 10 per cent.
salicylic.
Ammonium hydrate.
Potassium hydrate (sticks).
" 20 per cent. solution.
Sodium hydrate (sticks).
" 40 per cent. solution.
" deci-normal solution.
Silver nitrate, standard sol.
Copper sulphate, 1 to 10 sol.
Ammonium sulphate, sat. sol.
Sodium chlorid, sat. sol.
Litmus papers.
Ethyl alcohol.
Methyl alcohol.
Amylic alcohol.
Potassium chlorate, 1 per cent. sol.
Lead acetate (trebasic).
Sodium acetate.
Potassium ferrocyanid.
Zinc chlorid, 5 per cent. aqu. sol.
Potassium acetate.
Chloral.
Sodium carbonate.
Neutral ferric chlorid.
Barium chlorid, standard sol.
Sodium nitroprussid.
Solution hydrogen dioxid.
Bromine water.
Phenylhydrazin hydrochlorid.
Ethylene diamine hydate, 10 per cent. aqueous.
Zinc acetate, 10 per cent. alcoholic sol.
Phenolphthalein, 1 per cent. alcohol. sol.
Powdered guaiac.
Orcin.
Chloroform.
Lead carbonate.

FOR BLOOD.

Normal saline solution.
Benzol.
Xylol.
Chloroform.
Litmus paper, red and blue.
Canada balsam.
Xylol balsam.
Xylol dammar.
Ether.
½ per cent. acetic acid.
2½ per cent. potassium bichromate.

REAGENTS—FOR URINE.

MAGNESIA MIXTURE:—

Ammonium chlorid	1 part.
Magnesium sulphate	1 part.
Ammonia water	1 part.
Water	8 parts.

The salts are dissolved in the water and the ammonia water then added.

KNOP'S SOLUTION:—

Bottle "A"—Sodium hydrate sol.	1:25
Bottle "B"—Bromine	1 part.
KBr.	1 part.
Water	8 parts.

For the test add 1 part of the bromine solution to 15 or 20 parts of the sodium hydrate solution.

PURDY'S REAGENT:—

Potassium ferrocyanid	10 parts.
Strong acetic acid	10 parts.
Water	10 parts.

TANRET'S REAGENT:—

Dissolve 33.1 grams potassium iodid in 200 cubic centimeters of water. Add 13.5 grams powdered mercuric chlorid and water, stirring until the red precipitate first formed has been dissolved.

Dilute to 900 cubic centimeters with water and add 100 cubic centimeters strong acetic acid. Allow to stand twelve hours, and then decant from precipitate and use clear solution. This forms a solution of mercuric-potassium iodid in dilute acetic acid.

ESBACH'S REAGENT:—

Picric acid	10 grams.
Citric acid	20 grams.
Water	1000 c.c.

FEHLING'S REAGENT:—

The reagent consists of two solutions which are kept in separate bottles until mixed immediately before using.

Solution "A"—Copper sulphate	34.64	grams.
Water	500.00	c.c.
Solution "B"—Sodium-potassium tartrate	173.00	grams.
Sodium hydroxid	125.00	grams.
Water	500.00	c.c.

These solutions are used in equal parts for the test.

NYLANDER'S REAGENT:—

Bismuth subnitrate	2	grams.
Rochelle salt	4	grams.
Sodium hydroxid (8 per cent. sol.)	100	c.c.

TROMMER-SIMROCK REAGENT:—

Copper sulphate	2	grams.
Potassium hydroxid (5 per cent. sol.)	150	c.c.
Glycerin	15	c.c.
Distilled water	15	c.c.

PURDY'S REAGENT FOR SUGAR:—

Copper sulphate	4.72	grams.
Glycerin	38.00	c.c
Water	200.00	c.c.

These should be dissolved in the water by gentle heat.

Potassium hydroxid	23.50	grams.
Water	200.00	c.c.

Dissolve separately and then add to the copper solution.

When cold add:—

Ammonium hydroxid (strong)	450.00	c.c.
Water, q. s.	1000.00	c.c.

DIAZO REAGENT:—

This reagent consists of two solutions which are kept separate until mixed for the test.

Solution "A"—Sulphanilic acid	1.0	gram.
Hydrochloric acid (con.)	50.0	c.c.
Water	1000.0	c.c.
Solution "B"—Sodium nitrite	1.0	c.c.
Water	200.0	c.c.

Proportion for test: "A," 5 c.c. "B," 3 drops.

FOR BLOOD.

HAYEM'S DILUTING SOLUTION FOR COUNTING RED BLOOD-CELLS:—

Mercuric bichlorid	0.5 gram.
Sodium sulphate	5.0 grams.
Sodium chlorid	1.0 gram.
Aq. dest.	200.0 c.c.

TOISSON'S SOLUTION FOR SIMULTANEOUSLY COUNTING RED AND WHITE CELLS:—

Methyl violet	0.05 gram.
Neutral glycerin	30.00 c.c.
Aq. dest.	80.00 c.c.

Mix and add:—

Sodium chlorid	1.00 gram.
Sodium sulphate	8.00 gram.
Aq. dest.	80.00 gram.

Filter. Twelve minutes required to stain white blood-cells.

FIXING METHODS:—

1. Equal parts of absolute alcohol and ether. The specimen should be immersed in this for half to two hours. This method is particularly good for malarial parasites and degeneration in blood-cells.

2. Absolute alcohol for five minutes.

3. Flemming's solution:—

Chromic acid, 1 per cent.	15 parts.
Osmic acid, 2 per cent.	4 parts.
Glacial acetic acid	1 part.

The blood-specimens, as soon as they are made, before they have time to air-dry, are plunged into this solution and allowed to remain for ten minutes. They are then washed in running water for ten minutes and dried. This solution is very useful for demonstrating the chromatin of nuclei.

4. Vapors of formaldehyde.

5. Heat.

6. Wood alcohol. This is coming rapidly in favor, as it can be mixed with the stain, thus reducing the time of preparing specimens.

FOR GASTRIC ANALYSIS.

Uffleman's Reagent:—

Carbolic acid (4 per cent.).....10 c.c.
Water .....20 c.c.
Liquor ferri chloridi..... 1 drop.

This solution should be a clear amethyst color, and should be prepared fresh for use.

Lugol's Iodin Solution:—

Iodin ..... 1 part.
Potassium iodid ..... 2 parts.
Water .....50 parts.

Gunzberg's Phloroglucin Vanillin:—

Phloroglucin ..... 2 gms.
Vanillin ..... 1 gm.
Alcohol .....30 c.c.

This solution, if active, is pale yellow. It darkens and deteriorates with age, especially on exposure to light, so should be kept in colored bottles and made fresh from time to time.

FOR CEREBRO-SPINAL FLUID AND MILK.

Saturated aqueous solution of methyl violet. (R 5.)

Cream Testing Solutions:—

"A"—Amylic alcohol.....37 parts by volume.
Methyl alcohol.....13 parts by volume.
Hydrochloric acid.....50 parts by volume.

"B"—Sulphuric acid. sp. gr. 1832.

STAINS.

EHRLICH'S "TRIACID" STAIN:—

Orange G.	13.0-14.0 c.c.
Acid fuchsin	6.0- 7.0 c.c.
Distilled water	15.0 c.c.
Methyl green	25.5 c.c.
Alcohol	10.0 c.c.
Glycerin	10.0 c.c.

The three stains, orange G, acid fuchsin, and methyl green, are prepared in saturated aqueous solutions, and then mixed in the above amounts while being shaken thoroughly.

EOSIN AND METHYLENE-BLUE:—

Eosin .....0.5 per cent. in 70 per cent. alcohol.
Stain for a few minutes, wash, blot, and apply.
Methylene-blue .....1 per cent. aqueous.
Stain for a few minutes.

CHEZINSKY STAIN:—

Methylene-blue, sat. aq. sol.	40 c.c.
Eosin, 0.5 per cent. in 70 per cent. alcohol.	20 c.c.
Distilled water	40 c.c.

The specimens are fixed in absolute alcohol for from five to thirty minutes, and stained in a thermostat at 37° C. for from three to six hours.

HEMATOXYLIN-EOSIN (Ehrlich's mixture):—

Eosin (crystals)	0.5 gram.
Hematoxylin	2.0 gram.
Absolute alcohol, Distilled water, Glycerin, of each	100.0 gram.
Glacial acetic acid	10.0 gram.

Alum in excess.

This mixture should be allowed to stand for several weeks before it is ready for use. The specimens are stained in from half to two hours.

CARBOL GENTIAN-VIOLET:—

Gentian-violet, conc. alcoholic sol.	10.0 c.c.
Carbolic acid, 5 per cent. watery sol.	100.0 c.c.

GIEMSA IMPROVED STAIN:—

Azur II eosin ..... 3.0 gram.
Azur II ..... 0.8 gram.
Exsiccate, pulverize, and sift.
Dissolve in chemically pure glycerin at 60° C. ..... 250.0 c.c.
When solution is complete add:—
Methyl alcohol at temperature 60° C..... 250.0 c.c.

Shake well, allow to stand for twenty-four hours, then filter.

DECOLORIZING SOLUTIONS:—

I. Acetic acid, 0.5 to 5.0 per cent. watery solution.
II. Nitric acid, 20 to 30 per cent.
III. Acid alcohol:—
Sulphuric acid (conc.)..... 30 drops.
Alcohol (95 per cent.) ..... 50 c.c.
Water .....150 c.c.

LOEFFLER'S ALKALINE METHYLENE-BLUE:—

Concent. alcohol solution methylene-blue... 30.0 c.c.
Potassium hydrate ($\frac{1}{10000}$) .....100.0 c.c.

ZIEHL'S CARBOL-FUCHSIN:—

Fuchsin in substance ..... 1 gm.
Carbolic acid (cryst.) ..... 5 gm.
Alcohol (95 per cent.) ..... 10. c.c.
Distilled water .....100. c.c.

Or it may be prepared by adding to a 5 per cent. watery solution of carbolic acid a saturated alcoholic solution of fuchsin until a metallic luster appears on the surface of the liquid.

GABBOTT'S METHOD:—

A—Fuchsin ..... 1 gm.
Absolute alcohol ..... 10 c.c.
Carbolic acid (5 per cent.) ..... 100 c.c.
B—Methylene-blue ..... 2 gm.
Sulphuric acid (35 per cent. sol.)..... 100 c.c.

GRAM'S IODINE:—

Iodin	1 gm.
Potassium iodid	2 gm.
Distilled water	300 c.c.

KOCH-EHRLICH GENTIAN-VIOLET:—

Take distilled water, 100. cubic centimeters, and add anilin oil, drop by drop, until the solution has an opalescent appearance. The vessel containing the mixture should be thoroughly shaken after the addition of each drop. It is then filtered through moistened filter paper until the filtrate is clear. To 100 cubic centimeters of the filtrate add 10 cubic centimeters of absolute alcohol and 2 cubic centimeters of concentrated solution of gentian-violet.

UNNA'S ORCEIN STAIN:—

Orcein in substance	1 gm.
Hydrochloric acid	1 c.c.
Absolute alcohol	100 c.c.

REAGENTS FOR STAINING FLAGELLA:—

Mordant.

Tannic acid (20 ac. to 80 water)	10 c.c.
Ferric sulphate cold sat. sol.	5 c.c.
Fuchsin sat. watery sol.	1 c.c.

Adjuvants.

Sodium hydrate 1 per cent. aqueous solution.

Sulphuric acid (1 c.c. equal 1 c.c. of 1 per cent. NaOH).

PAPPENHEIM'S SOLUTION:—

Corallin	1 part.
Absolute alcohol	100 parts.

Add to the above solution methylene-blue in bulk to saturation. Finally add 20 parts of glycerin.

LOEFFLER'S MORDANT:—

Tannic acid solution (2 parts acid, 80 parts water)	10. c.c.
Ferrous sulphate saturated solution	5. c.c.
Fuchsin saturated aqueous solution	1. c.c.

EBNER'S FLUID:—

Hydrochloric acid	2.5 c.cm.
Sodium chlorid, C. P.	2.5 c.cm.
Distilled water	100.0 c.cm.
Alcohol, 95 per cent.	500.0 c.cm.

UNIVERSAL STAINING METHOD:—

The discovery of the process whereby two or more colors could be chemically combined in one staining reagent, marked a great advance in the field of hematology. It affords greater opportunity for more detailed study of the minute structure of the cells of the blood, which in turn has resulted in a better classification of the different elements through the differentiation of new and distinct varieties which, until recently, have been unrecognized. Of these combination stains the Polychrome or "universal" staining method is by far the best and most practical, and is now rapidly superseding the older and more cumbersome methods. For this reason it becomes a matter of considerable importance, almost a necessity, that the clinical worker should have at hand one or more of these stains ready for immediate use when occasion requires.

Unfortunately the preparation of this class of stain involves considerable expenditure of time, and demands no small amount of chemical knowledge and manipulative skill. These factors combine to limit the preparation of these stains to a comparatively small number of experienced workers, while the majority of the profession is dependent upon unstable liquid stains, obtainable through the supply houses, the composition of which is frequently so variable as to render the results valueless. Further, all aniline stains of this character are prone to decomposition when kept even for a moderately long time in solution.

Fortunately the demand for a uniform and reliable stain has recently been met by a London Pharmaceutical House,[2] who now carry in stock a number of very uniform and perfectly reliable stains under the name of "Soloid" brand. These stains are very carefully prepared and their composition practically

[2] Burroughs Wellcome & Co., with a branch at 45 Lafayette Street, New York City.

uniform. A definite quantity of the dried stain is compressed into a tiny tablet and dispensed in vials containing six. Each package is accompanied with information indicating the proper dilution and best working conditions for that particular stain.

The "Soloid" stains permit of the preparation of small quantities of liquid stain whereby waste through evaporation or decomposition is reduced to a minimum, at the same time the results obtained, as far as the author has employed them, are in every way satisfactory.

The following is abstracted from the last descriptive catalogue of the above company:—

The majority of the stains are employed in alcohol in solution, and the different alcohols commonly used are here described.

Absolute Alcohol contains not less than 99 per cent. by weight of pure ethyl alcohol, C_2H_5OH.

Alcohol of a Stated Percentage, *e.g.*, 50 per cent. alcohol, means a mixture with water which contains the stated percentage, *i.e.*, 50 per cent. by volume of pure ethyl alcohol.

Methyl Alcohol is a pure substance, CH_3OH, prepared by the purification of commercial wood spirit. Commercial methyl alcohol, which is impure, must not be employed in the preparation of Jenner's, Leishman's, or Romanowski stains. .

The amounts of distilled water and absolute alcohol respectively, required to produce saturated solution of certain dyes in common use, are indicated in the following table:—

	"Soloid" product of 0.1 gm. of dye	Water (c.c.)	Alcohol (c.c.)
Bismarck Brown, pure	1	7	7
Fuchsin, "	1	10	2.5
Gentian-Violet, "	1	7	7
Hematoxylin, "	1	2	1
Methyl Violet, "	1	5	1
Methylene-Blue, "	1	7	7
Thionin Blue, "	1	5	10

Aqueous dilutions of the above, containing 5 to 10 per cent. of these saturated solutions, are well adapted for ordinary staining purposes. Various other solutions, ready for immediate use, may be prepared from "Soloid" Microscopic Stains according to the following directions:—

Eosin.—To obtain a solution of eosin suitable for general staining, one "Soloid" product may be dissolved in 20 cubic centimeters of 50 per cent. alcohol. This gives a 0.5 per cent. solution.

Loeffler's Alkaline Methylene-Blue.—Dissolve one "Soloid" methylene-blue in 7 cubic centimeters of absolute alcohol, and add 25 cubic centimeters of distilled water, to which one drop of *Liquor Potassae U.S.P.* has been added.

Aniline Gentian-Violet.—Dissolve one "Soloid" gentian-violet in 7 cubic centimeters of absolute alcohol, and add 63 cubic centimeters of a freshly filtered saturated solution of aniline oil in distilled water.

Carbol Gentian-Violet.—Dissolve one "Soloid" gentian-violet in 7 cubic centimeters of absolute alcohol, and add 63 cubic centimeters of a 1 per cent. aqueous solution of carbolic acid.

Ziehl's Carbol-Fuchsin.—Thoroughly powder and dissolve one "Soloid" fuchsin in 3 cubic centimeters of absolute alcohol, add 22 cubic centimeters of 5 per cent. aqueous solution of carbolic acid; shake well, and filter before using.

Gram's Iodine Solution.—Dissolve one "Soloid" product of reagent A in 10 cubic centimeters of distilled water, add one of reagent B, and when solution is complete, dilute to 15 cubic centimeters with distilled water.

Carbol Thionin Blue.—Dissolve one "Soloid" thionin blue in 100 cubic centimeters of a 5 per cent. aqueous solution of carbolic acid.

Borax Methylene-Blue.—Dissolve one "Soloid" borax methylene-blue in 10 cubic centimeters of distilled water.

Delafield's Hematoxylin.—Dissolve one "Soloid" hematoxylin (Delafield) in 10 cubic centimeters of a 25 per cent. solution of glycerin in water.

Eosin-Azur (for Giemsa staining with one solution).—Dissolve one "Soloid" product in 5 cubic centimeters of *pure* methyl alcohol.

Eosin-Methylene-Blue (Louis Jenner's Stain).—Dissolve one "Soloid" product in 10 cubic centimeters of *pure* methyl alcohol.

Romanowsky Stain (Leishman's Modification).—Dissolve one "Soloid" product in 10 cubic centimeters of *pure* methyl alcohol.

Sodium Carbonate.—When employed in the preparation of Romanowsky stain, dissolve one "Soloid" product (0.05 gram) in 10 cubic centimeters of distilled water, and add one "Soloid" methylene-blue, 0.1 gram (for the method of preparation and use, see *British Medical Journal*, September 21, 1901, page 757).

Biondi-Ehrlich-Heidenhain Triple Stain.—Dissolve one "Soloid" Ehrlich triple stain in 25 cubic centimeters of distilled water; one "Soloid" acid fuschin in 2 cubic centimeters of distilled water, and mix. The mixture is ready for use and keeps well.

Toison Blood Fluid.—For the preservation of blood corpuscles and the counting of the same. Dissolve one "Soloid" product in 3 cubic centimeters of glycerin and 16 cubic centimeters of distilled water. The solution should always be filtered immediately before use.

The Polychrome Methylene-Blue-Eosin Stains (Romanowski).—There are about fifteen different modifications of this stain. The majority of them are difficult of manufacture, even by an expert, so it is recommended that they be bought ready-made from the laboratory apparatus and supply houses which make them.

For those who desire to make this stain for themselves, the following modification by Hastings is appended, as one which is comparatively simple of manufacture.[3]

All the Romanowski stains are made with wood alcohol, which, during the first portion of its application, acts as a fixative.

Hastings' Stain.—The dry stains necessary are eosin (water solution) yellow (Grubler), and methylene-blue (Ehrlich's rectif.) (Grubler).

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[3] Hastings: Johns Hopkins Hospital Bulletin, 1905.

Solution "A"—Eosin 1 per cent. aqueous.

Solution "B"—Alkaline methylene-blue 1 per cent. aqueous.

Solution "C"—Methylene-blue 1 per cent. aqueous.

Solution "A" may be kept ready-made, solutions "B" and "C" must be made fresh.

To prepare solution "B," use a warm 1 per cent. solution of dry powdered sodium carbonate. Add to it one per cent. of methylene-blue powder, and heat over a water bath for 15 minutes. Add 30 cubic centimeters of water for each 100 cubic centimeters of the original fluid, and heat again for 15 minutes. Then pour off the solution from the residue and divide into two equal parts. To one part add enough 12.5 per cent. acetic acid solution to make a faintly acid reaction. This is best determined by taking a piece of blue litmus paper and allowing a drop to fall upon it, taking as the end reaction the point at which the margin of the drop after absorption in the paper shows a faint pink. Then add the remaining unneutralized portion to this.

To mix the stain use distilled water 1000 cubic centimeters. Solution "A," 100 cubic centimeters; solution "B," 200 cubic centimeters; solution "C," 70 to 80 cubic centimeters. In adding solution "C" put in 70 cubic centimeters at once, and stir well; if no precipitate appears, add 1 cubic centimeter at a time until one does appear. After the precipitate appears the stain is allowed to stand for half an hour and then filtered through one filter. Forced filtration is generally necessary.

The dry residue is removed from the paper and pulverized. It may be kept in this form or dissolved in Merck's pure methyl alcohol. Seven- to nine-tenths of a gram of dried stain is usually obtained. Three-tenths of a gram is dissolved in 100 cubic centimeters of alcohol for the staining solution. In dissolving the stain it must be rubbed up with the alcohol in a mortar, as the powder is with difficulty soluble.

If there are more than nine-tenths of a gram of the dried stain obtained, the preparation is useless, and should be begun again.

For each new lot of stain made up, one must determine the relative proportion of stain and water used in staining, and the relative lengths of time during which the pure and diluted stain is allowed to act.

Usually two drops of stain on the smear for one minute and then four drops of water added and allowed to act for four minutes gives the best result.

For uniformity in dropping a dropper should be used.

All polychrome methylene-blue stains require experiment, since different mixtures by the same method require slight variations in their use. These must be ascertained by trial. Use distilled water to wash the specimen, since tap-water may ruin it.

Multiples of a Grain

From 1 grain to 1 ounce

U. S. A.	Metric	U. S. A.	Metric
gr. 1	0.065 gm.	gr. 15	0.972 gm.
gr. 1⅓	0.086 gm.	gr. 18	1.166 gm.
gr. 1½	0.097 gm.	gr. 20	1.296 gm.
gr. 1¾	0.113 gm.	gr. 25	1.620 gm.
gr. 2	0.13 gm.	gr. 30	1.944 gm.
gr. 2½	0.162 gm.	gr. 35	2.268 gm.
gr. 3	0.194 gm.	gr. 40	2.592 gm.
gr. 3½	0.227 gm.	gr. 50	3.24 gm.
gr. 4	0.259 gm.	gr. 60	3.89 gm.
gr. 5	0.324 gm.	gr. 120	7.78 gm.
gr. 6	0.389 gm.	oz. ⅛	3.54 gm.
gr. 7	0.454 gm.	oz. ¼	7.08 gm.
gr. 8	0.518 gm.	oz. ½	14.17 gm.
gr. 8¾	0.567 gm.	dr. 4	15.55 gm.
gr. 9	0.583 gm.	oz. 1	28.35 gm.
gr. 10	0.648 gm.	dr. 8	31.1 gm.
gr. 12	0.778 gm.		

Equivalents of U. S. A. and Metric Measures of Capacity

From half-a-minim to 1 fluid ounce

U. S. A.	Metric	U.S.A.	Metric
min. ½	0.03 c.c.	min. 20	1.232 c.c.
min. 1	0.062 c.c.	min. 25	1.54 c.c.
min. 2	0.123 c.c.	min. 30	1.848 c.c.
min. 3	0.185 c.c.	min. 35	2.156 c.c.
min. 4	0.246 c.c.	min. 40	2.464 c.c.
min. 5	0.308 c.c.	min. 50	3.08 c.c.
min. 6	0.370 c.c.	min. 60	3.70 c.c.
min. 7	0.431 c.c.	min. 90	5.54 .c.
min. 8	0.493 c.c.	min. 120	7.39 c.c.
min. 9	0.554 c.c.	min. 180	11.09 c.c.
min. 10	0.616 c.c.	min. 240	14.79 c.c.
min. 12	0 739 c.c.	min. 360	22.18 c.c.
min. 15	0 924 c.c.	min. 480	29.57 c.c.

In Continental prescribing, a smaller quantity than half a cubic centimeter is usually expressed in drops, which, in dispensing, are dropped from pipette into the cubic centimeter measure.

Approximate U. S. A. Equivalents of Metric Measure of Capacity

Metric	U. S. A.
1 c.c.	16 (16.23) min.
2 c.c.	32½ min.
3 c.c.	48¾ min.
4 c.c.	1 fl. dr. 5 min.
5 c.c.	1 fl. dr. 21 min.
6 c.c.	1 fl. dr. 37 min.
7 c.c.	1 fl. dr. 54 min.
8 c.c.	2 fl. dr. 10 min.
9 c.c.	2 fl. dr. 26 min.
10 c.c.	2 fl. dr. 42 min.
12 c.c.	3 fl. dr. 23 min.
15 c.c.	4 fl. dr. 4 min.
20 c.c.	5 fl. dr. 25 min.

Metric	U. S. A.
25 c.c.	6 fl. dr., 46 min.
30 c.c.	8 fl. dr., 7 min.
40 c.c.	1 fl. oz., 2 fl. dr., 49 min.
50 c.c.	1 fl. oz., 5 fl. dr., 32 min.
75 c.c.	2 fl. oz., 4 fl. dr., 17 min.
100 c.c.	3 fl. oz., 3 fl. dr., 3 min.
125 c.c.	4 fl. oz., 1 fl. dr., 49 min.
150 c.c.	5 fl. oz., 0 fl. dr., 35 min.
200 c.c.	6 fl. oz., 6 fl. dr., 6 min.
300 c.c.	10 fl. oz., 1 fl. dr., 9 min.
500 c.c.	16 fl. oz., 7 fl. dr., 15 min.
1 litre	33 fl. oz., 6 fl. dr., 31 min.

Approximate U. S. A. Equivalents of Metric Measures of Mass

Metric	U. S. A.
1 mgm	1/64 gr.
2 mgm	1/33 gr.
3 mgm	1/21 gr
4 mgm	1/16 gr
5 mgm	1/13 gr.
6.5 mgm	1/10 gr.
8 mgm	⅛ gr.
1 cgm	1/6 gr.
2 cgm	⅓ gr.
3 cgm	½ gr.
5 cgm	¾ gr.
6.5 cgm	1 gr.
10 cgm	1½ gr.
15 cgm	2¼ gr.
20 cgm	3 gr.
26 cgm	4 gr.
30 cgm	4½ gr.
40 cgm	6¼ gr.
50 cgm	7¾ gr.
75 cgm	11½ gr.

Metric	U. S. A.
1 gm	15½ (15.432) gr.
2 gm	30⅞ gr.
3 gm	46¼ gr.
4 gm	61¾ gr.
5 gm	77⅙ gr.
7.5 gm	115¾ gr.
10 gm	154⅓ gr.
15 gm	231½ gr.
20 gm	308⅗ gr.
25 gm	385⅘ gr.
30 gm	1 oz. 25½ gr.
40 gm	1 oz. 179⅕ gr.
50 gm	1 oz. 334 gr.
75 gm	2 oz. 282½ gr.
100 gm	3 oz. 230¾ gr.
150 gm	5 oz. 127½ gr.
250 gm	8 oz. 358 gr.
500 gm	1 lb. 1 oz. 278 gr.
750 gm	1 lb. 10 oz. 200 gr.
1 kgm	2 lb. 3 oz. 120 gr.

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